# The waveTiling package

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### 1 Introduction

In this waveTiling package vignette the package's main functionalities to conduct a tiling array trancriptome analysis are illustrated. The package contains an implementation of the basic wavelet-based functional model introduced in [Clement et al., 2012], and its extensions towards more complex designs described in [De Beuf et al., 2012]. The leaf development data set [Andriankaja et al., 2012] contains genome-wide expression data measured for six developmental time points (day 8 to day 13) on the plant species Arabidopsis thaliana. The experiment was conducted with AGRONOMICS1 tiling arrays [Rehrauer et al., 2010] and contains three biological replicates per time point.

## 2 Read in and prepare data for analysis

First we have to load the wave Tiling package and the wave Tiling Data package. The latter contains an Tiling Feature Set (leafdev) from the oligo Classes package with the expression values for the leaf development experiment, and the TAIR 9 Arabidopsis thaliana gene identifier data tair 9gff. Make sure to also load the pd. atdschip. tiling wich contains the tiling array info to map the probe locations on the array to the exact genomic positions. The pd. atdschip. tiling package was created by using the pdInfoBuilder package, which should also be used to build similar packages for other array designs.

- > library(waveTiling)
- > library(waveTilingData)
- > library(pd.atdschip.tiling)
- > data(leafdev)
- > data(tair9gff)

We first change the class to WaveTilingFeatureSet, which is used as input for the wavelet-based transcriptome analysis, and add the phenotypic data for this experiment.

```
> leafdev <- as(leafdev, "WaveTilingFeatureSet")</pre>
> leafdev <- addPheno(leafdev,noGroups=6,
          groupNames=c("day8","day9","day10","day11","day12","day13"),
          replics=rep(3,6)
> leafdev
WaveTilingFeatureSet (storageMode: lockedEnvironment)
assayData: 6553600 features, 18 samples
  element names: exprs
protocolData
 rowNames: caquinof_20091023_S100_v4.CEL
    caquinof_20091023_S101_v4.CEL ...
    caquinof_20091023_S117_v4.CEL (18 total)
  varLabels: exprs dates
  varMetadata: labelDescription channel
phenoData
 rowNames: day8.1 day8.2 ... day13.3 (18 total)
  varLabels: group replicate
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: pd.atdschip.tiling
```

Before starting the transcriptome analysis, the probes that map to several genomic locations (either PM or MM, or forward and reverse strand) are filtered using filterOverlap. This function can also be used if the probes have to be remapped to another version of the genome sequence as the version used for the array design. For instance, the probes on the AGRONOMICS1 array are build based on the TAIR 8 genome, and remapped onto the TAIR 9 sequence. The function needs an argument BSgenomeObject available from loading the appropriated BSgenome package. The output is an object of class mapFilterProbe. After filtering and/or remapping, the expression data are background-corrected and quantile-normalized (bgCorrQn). The mapFilterProbe leafdevMapAndFilterTAIR9 is used to make sure only the filtered probes are used in the background correction and normalization step.

```
> library(BSgenome.Athaliana.TAIR.TAIR9)
> ### temporary during development
> # leafdevMapAndFilterTAIR9 <- filterOverlap(leafdev,remap=TRUE,
           BSgenomeObject=Athaliana,chrId=1:7,
> #
           strand="both",MM=FALSE)
> load("/home/kdebeuf/research/tiling/leafdevMapAndFilterTAIR9.rda")
> ###
> leafdevMapAndFilterTAIR9
Remapped and filtered probe information
No. of filtered probes: 5894070
> ### temporary during development
> # leafdevBQ <- bgCorrQn(leafdev,useMapFilter=leafdevMapAndFilterTAIR9)
> load("/home/kdebeuf/research/tiling/leafdevBQ.rda")
> ###
> leafdevBQ
WaveTilingFeatureSet (storageMode: lockedEnvironment)
assayData: 5894070 features, 18 samples
```

```
element names: exprs
protocolData
rowNames: caquinof_20091023_S100_v4.CEL
caquinof_20091023_S101_v4.CEL ...
caquinof_20091023_S117_v4.CEL (18 total)
varLabels: exprs dates
varMetadata: labelDescription channel
phenoData
rowNames: day8.1 day8.2 ... day13.3 (18 total)
varLabels: group replicate
varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: pd.atdschip.tiling
```

### 3 Wavelet-based transcriptome analysis

#### 3.1 Standard analysis flow

The analysis has to be conducted in a chromosome- and strand-wise manner. First, the wavelet-based model is fitted to the expression data, leading to a *WfmFit*-class object leafdevFit.

```
> chromosome <- 1
> strand <- "forward"
> leafdevFit <- wfm.fit(leafdevBQ,filter.overlap=leafdevMapAndFilterTAIR9,
          design="time", n.levels=10,
          chromosome=chromosome,strand=strand,
          var.eps="marg",prior="improper",skiplevels=1,
          save.obs="plot",trace=TRUE)
Gauss Seidel algorithm...
 iteration 1 of 20
 iteration 2 of 20
 iteration 3 of 20
 iteration 4 of 20
 iteration 5 of 20
 iteration 6 of 20
 Gauss Seidel algorithm converged
> leafdevFit
Fitted object from wavelet based functional model - Time Design
Wavelet filter used: haar
Number of wavelet decomposition levels: 10
Number of probes used for estimation: 753664
Genome Info :
        Chromosome: 1
        Strand: forward
       Minimum probe position: 50
        Maximum probe position: 30397589
```

If the redundant probes have been filtered using filterOverlap the resulting mapFilterProbe class object should be given as an argument filter.overlap, to ensure that the expression values are properly linked to the genomic information such as chromosome and strand. In this analysis we use a time-course design (design). The number of levels in the wavelet decomposition is 10 (n.levels). We use marginal maximum likelihood to estimate the residual variances (var.eps) and put an improper prior (prior) on the effect functions (see [Clement et al., 2012]).

Next, the WfmFit-class object leafdevFit is used as input for the inference function wfm.inference. This function outputs the WfmInf-class object leafdevInf from which transcriptionally active regions of interests, given a chosen threshold value, can be extracted.

```
> delta <- log(1.2,2)
> leafdevInfCompare <- wfm.inference(leafdevFit,
+ contrasts="compare",delta=c("median",delta))
> leafdevInfCompare
object of class 'WfmInf'
```

The contrasts argument is used to indicate the type of inference analysis one wants to conduct, e.g. compare to detect differentially expressed regions between the different time points. By default, transcriptionally active regions based on the mean expression over all arrays are also given in the output. With the delta the threshold value to use in the statistical tests can be set. It is a vector with as first element the threshold for the overall mean trancript discovery. This is taken to be the median of the expression values over all arrays in this case. The second element is the threshold for the differential expression analysis. This threshold is equal for each pairwise comparison if the length of delta is 2. If one wants to use different thresholds the length of delta must be r+1 with r the number of pairwise comparisons, where each element is associated with an individual threshold value.

Much information is stored in the WfmFit-class and WfmInf-class objects. Primarily, we are interested in the genomic regions that are significantly transcriptionally affected according to the research question of interest.

- > sigGenomeRegionsCompare <- getGenomicRegions(leafdevInfCompare)
  > sigGenomeRegionsCompare[[2]]
- IRanges of length 439

	start	end	width
[1]	118321	118673	353
[2]	145361	145457	97
[3]	163985	164081	97
[4]	219441	219537	97
[5]	219985	220081	97
[6]	220177	220561	385
[7]	220657	220817	161
[8]	220913	221009	97
[9]	312497	312977	481
[431]	29550740	29550836	97
[432]	29916020	29916116	97
[433]	30005524	30005620	97
[434]	30027828	30027924	97
[435]	30040788	30040884	97
[436]	30216949	30217109	161
[437]	30217333	30217429	97

```
[438] 30217909 30218005 97
[439] 30219061 30219157 97
```

> length(sigGenomeRegionsCompare)

[1] 16

The getGenomicRegions accessor outputs a list of IRanges objects denoting the start and end position of each significant region. The first element in the list always gives the significant regions for the mean expression over all arrays (transcript discovery). Elements 2 to 16 in sigGenomeRegions give the differentially expressed regions between any pair of contrasts between different time points. The order is always 2-1, 3-1, 3-2, 4-1,... Hence, sigGenomeRegions[[2]] gives the differentially expressed regions between time point 2 and time point 1.

If an annotation file containing gene identifiers is available, we can extract both significantly affected genes with getSigGenes, and the non-annotated regions with getNonAnnotatedRegions. Both functions output a *list* of *GRanges* objects.

> sigGenesCompare <- getSigGenes(fit=leafdevFit,inf=leafdevInfCompare,annoFile=tair9gff)

find overlaps with detected regions...

> head(sigGenesCompare[[2]])

GRanges with 6 ranges and 6 elementMetadata values:

	seqnames		ranges	strand	feature	id
	<rle></rle>	<	IRanges>	<rle>  </rle>	<character></character>	<character></character>
[1]	1	[116943,	118764]	+	gene	AT1G01300
[2]	1	[143564,	145684]	+	gene	AT1G01370
[3]	1	[163419,	166239]	+	gene	AT1G01448
[4]	1	[218994,	221286]	+	gene	AT1G01600
[5]	1	[218994,	221286]	+	gene	AT1G01600
[6]	1	[218994,	221286]	+	gene	AT1G01600
	regNo	perc0ve	rGene per	rcOverReg	totPercOver(	Gene

	<integer></integer>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>
[1]	1	19.374314	100	19.374314
[2]	2	4.573314	100	4.573314
[3]	3	3.438497	100	3.438497
[4]	4	4.230266	100	36.502399
[5]	5	4.230266	100	36.502399
[6]	6	16.790231	100	36.502399

seqlengths:

1 2 3 4 5 6 7 NA NA NA NA NA NA NA

> nonAnnoCompare <- getNonAnnotatedRegions(fit=leafdevFit,inf=leafdevInfCompare,

+ annoFile=tair9gff)

get annotated regions...
find overlaps with detected regions...

> head(nonAnnoCompare[[2]])

GRanges with 6 ranges and 0 elementMetadata values:

```
seqnames
                    ranges strand
       <Rle>
                 <IRanges>
                             <Rle>
[1]
            1 [ 113,
                       241]
[2]
            1 [ 338,
                      977]
[3]
            1 [1585, 1713]
[4]
            1 [1810, 2097]
[5]
            1 [2289, 2834]
[6]
            1 [3121, 3281]
seqlengths:
 1
NA
```

Using the same *WfmFit*-object leafdevFit, we can run the analysis to analyze transcriptional time effects (leafDevInfTimeEffect) and have look at time-wise transcriptionally active regions (leafdevInfMeans).

Besides the available standard design analyses given by the design argument in the wfm.fit function and the contrasts argument in the wfm.inference, it is also possible to provide custom design and contrast matrices in the wave Tiling package. This custom design is illustrated based on the polynomial contrast matrix used in a time-course analysis.

```
> custDes <- matrix(0,nrow=18,ncol=6)</pre>
> orderedFactor <- factor(1:6,ordered=TRUE)</pre>
> desPoly <- lm(rnorm(6)~orderedFactor,x=TRUE)$x</pre>
> custDes[,1] <- 1
> custDes[,2:6] <- apply(desPoly[,2:6],2,rep,getReplics(leafdevBQ))</pre>
> custDes
                [,2]
                          [,3]
                                     [,4]
                                               [,5]
      [,1]
                                                           [,6]
 [1,]
        1 -0.5976143  0.5455447 -0.3726780
                                          0.1889822 -0.06299408
 [2,]
        1 -0.5976143  0.5455447 -0.3726780
                                          0.1889822 -0.06299408
 [3,]
        [4,]
        1 -0.3585686 -0.1091089
                               0.5217492 -0.5669467
                                                     0.31497039
 [5,]
        1 -0.3585686 -0.1091089
                               0.5217492 -0.5669467
                                                     0.31497039
 [6,]
        1 -0.3585686 -0.1091089
                               0.5217492 -0.5669467
                                                     0.31497039
 [7,]
        1 -0.1195229 -0.4364358
                               0.2981424
                                          0.3779645 -0.62994079
 [8,]
        1 -0.1195229 -0.4364358
                               0.2981424
                                          0.3779645 -0.62994079
 [9,]
        [10,]
          0.1195229 -0.4364358 -0.2981424 0.3779645
                                                    0.62994079
[11,]
        1 0.1195229 -0.4364358 -0.2981424
                                          0.3779645
                                                     0.62994079
[12,]
          0.1195229 -0.4364358 -0.2981424 0.3779645
                                                     0.62994079
[13,]
        1 0.3585686 -0.1091089 -0.5217492 -0.5669467 -0.31497039
[14,]
        1 0.3585686 -0.1091089 -0.5217492 -0.5669467 -0.31497039
[15,]
          0.3585686 -0.1091089 -0.5217492 -0.5669467 -0.31497039
[16,]
        1 0.5976143 0.5455447
                               0.3726780
                                          0.1889822
                                                    0.06299408
[17,]
          0.5976143  0.5455447  0.3726780  0.1889822  0.06299408
[18,]
        1 0.5976143 0.5455447 0.3726780 0.1889822 0.06299408
```

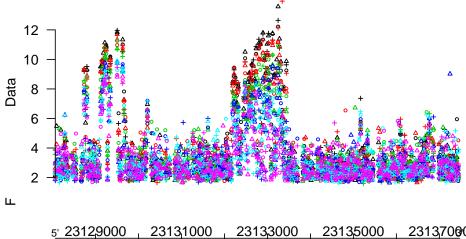
```
> leafdevFitCustom <- wfm.fit(leafdevBQ,filter.overlap=leafdevMapAndFilterTAIR9,
         design="custom",design.matrix=custDes,n.levels=10,
         chromosome=chromosome, strand=strand, var.eps="marg",
         prior="improper", skiplevels=1, save.obs="plot", trace=TRUE)
Gauss Seidel algorithm...
iteration 1 of 20
iteration 2 of 20
iteration 3 of 20
iteration 4 of 20
iteration 5 of 20
iteration 6 of 20
Gauss Seidel algorithm converged
> noGroups <- getNoGroups(leafdevBQ)
> myContrastMat <- matrix(0,nrow=noGroups*(noGroups-1)/2,ncol=noGroups)</pre>
> hlp1 <- rep(2:noGroups,1:(noGroups-1))</pre>
> hlp2 <- unlist(sapply(1:(noGroups-1),function(x) seq(1:x)))</pre>
> for (i in 1:nrow(myContrastMat))
+ {
+
         myContrastMat[i,hlp1[i]] <- 1</pre>
         myContrastMat[i,hlp2[i]] <- -1</pre>
+ }
> myContrastMat
     [,1] [,2] [,3] [,4] [,5] [,6]
 [1,]
       -1
            1
                  0
                            0
                                 0
                       0
 [2,]
       -1
             0
                  1
                       0
                            0
                                 0
 [3,]
        0
            -1
                  1
                       0
                            0
                                 0
 [4,]
       -1
            0
                  0
                       1
                            0
                                 0
 [5,]
       0
            -1
                            0
                                 0
                0
                     1
 [6,]
       0
           0
                -1
                            0
                              0
                      1
 [7,]
       -1
            0
                  0
                       0
                            1
                                 0
                0
                     0
                          1
 [8,]
        0
            -1
                                 0
 [9,]
      0
           0
                -1
                     0 1
[10,]
                0 -1 1
       0
            0
                                 0
[11,]
       -1
            0
                 0
                       0
                            0
                0
                          0 1
[12,]
      0
           -1
                      0
[13,]
           0
                 -1
                      0
                            0 1
                     -1
[14,]
        0
             0
                 0
                            0
                                 1
[15,]
> leafdevInfCustom <- wfm.inference(leafdevFitCustom,contrast.matrix=myContrastMat,
         delta=c("median", log(1.2,2)))
```

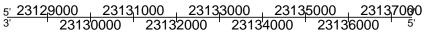
#### 3.2 Plot function

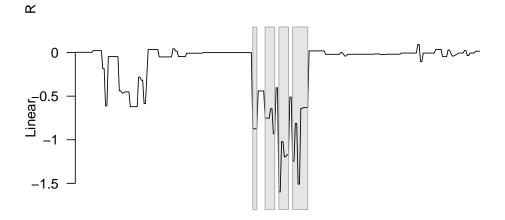
Plots can be made very easily using the plot function which needs both the WfmFit- and WfmInf-class objects as input. It also needs an appropriate annotation file.

```
> gene1 <- tair9gff[tair9gff$ID %in% "AT1G04350",]
> start <- gene1$start-2500</pre>
```

```
> end <- gene1$end+2500
> plot(fit=leafdevFit,inf=leafdevInfCompare,
           annoFile=gene1,minPos=start,maxPos=end,
           two.strand=TRUE,plotData=TRUE,
           plotMean=FALSE, tracks=c(1,2,6,10,11))
      10
       8
   Data
       6
  ш
          \begin{smallmatrix} 18 \hline 463000 & 1164000 & 1165000 & 1166000 & 1167000 & 1168000 & 116909 \end{smallmatrix}
  \alpha
  Gr2-Gr1
       6
       4
       2
       0
  Gr3-Gr1
       6 -
       4
       2
       0
  Gr4-Gr3
       6 -
       4
       2
       0
  Gr5-Gr4
       6 -
       4
       2
       0
  Gr6-Gr1
       6
       4
       2
> gene2 <- tair9gff[tair9gff$ID %in% "AT1G62500",]</pre>
> start <- gene2$start-4000
> end <- gene2$end+4000
> plot(fit=leafdevFit,inf=leafdevInfTimeEffect,
           annoFile=gene2,minPos=start,maxPos=end,
           {\it two.strand=TRUE,plotData=TRUE,}
           plotMean=FALSE, tracks=1)
```

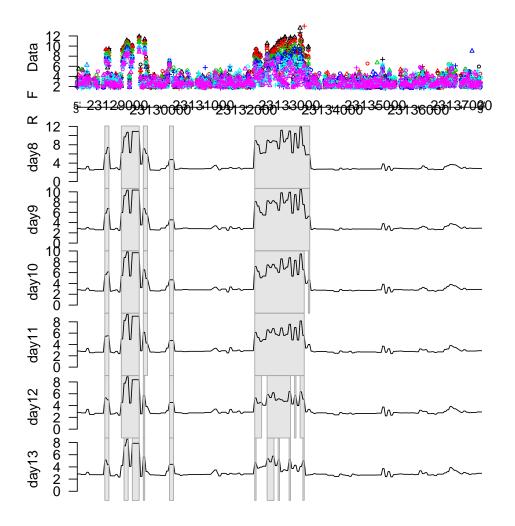






> plot(fit=leafdevFit,inf=leafdevInfMeans,

- annoFile=gene2,minPos=start,maxPos=end,
- two.strand=TRUE,plotData=TRUE,
- plotMean=FALSE,tracks=1:6)



### 3.3 Accessor functions

There are a number of accessor functions available that are not necessarily needed to run a standard trancriptome analysis, but still can extract useful information from the *WfmFit*- and *WfmInf*-class objects. Some of the more interesting ones are illustrated below. For a complete overview, consult the package's help pages.

#### > getGenomeInfo(leafdevFit)

> dataOrigSpace[1:8,1:8]

18 753664

[1]

```
307998
                  698838
                           619075
                                    234395
                                            361615
                                                     533643
day8.1 2.174546 3.052743 2.138756 2.367355 2.138756 5.000700 2.558657
day8.2 1.814251 2.328446 4.165652 3.734485 2.892113 5.173654 3.805115
day8.3 2.803171 2.196780 3.676953 3.380027 3.616066 3.798155 2.536061
day9.1 2.006381 2.176768 3.484187 2.773344 3.184647 5.014818 3.542103
day9.2 2.280221 2.486904 2.860395 3.865426 2.380023 4.238844 2.536061
day9.3 2.834020 2.600848 2.550766 2.954089 3.390393 4.836999 2.655298
day10.1 3.070930 2.857383 3.602468 2.803171 2.702066 6.216891 3.359889
day10.2 2.213182 2.957153 3.825710 3.181483 2.380023 3.667084 2.380023
         735748
day8.1 3.132112
day8.2 2.146153
day8.3 2.695701
day9.1 2.416558
day9.2 4.140161
day9.3 2.550766
day10.1 4.406534
day10.2 2.213182
> dataWaveletSpace <- getDataWaveletSpace(leafdevFit)</pre>
> dim(dataWaveletSpace)
       18 753664
[1]
> dataWaveletSpace[1:8,1:8]
             [,1]
                        [,2]
                                  [,3]
                                              [,4]
                                                         [,5]
day8.1
        0.6209791 \quad 0.1616440 \ \ 2.0236997 \quad 0.40549360 \quad 0.6196431
        0.3635910 -0.3048809 1.6132925 -1.17306372 -0.4977551
day8.3 -0.4287828 -0.2099587 0.1287563 0.11288256 -0.9997125
day9.1
        0.1204821 - 0.5026421 \ 1.2941265 - 0.79588033 \ 0.1552622
day9.2
        day9.3 -0.1648775 0.2851923 1.0229051 -0.07391526 -0.5789659
day10.1 -0.1510001 -0.5651883 2.4853564 0.74008982 0.3803080
day10.2 0.5260667 -0.4555375 0.9100900 -0.11797403 -0.5671088
              [,6]
                          [,7]
                                     [,8]
dav8.1
        0.54089690 0.99127802 0.5479831
day8.2 -0.08285642 0.83747549 -0.1653711
day8.3
        0.76416907 0.15395435 0.3799351
day9.1
        day9.2 0.03655212 0.53390384 -1.4254594
day9.3 -0.40229994 1.50563770 -0.1675201
day10.1 1.97672325 -0.08831259 0.8829002
day10.2 -0.43654967 -0.19902948 0.1085624
> getDesignMatrix(leafdevFit)
                [,2]
                           [,3]
                                      [,4]
                                                 [,5]
 [1,]
        1 \ -0.5976143 \quad 0.5455447 \ -0.3726780 \quad 0.1889822 \ -0.06299408
 [2,]
        1 -0.5976143  0.5455447 -0.3726780  0.1889822 -0.06299408
 [3.]
        1 -0.5976143  0.5455447 -0.3726780  0.1889822 -0.06299408
 [4,]
        1 -0.3585686 -0.1091089 0.5217492 -0.5669467 0.31497039
```

```
Γ5. ]
        1 -0.3585686 -0.1091089 0.5217492 -0.5669467 0.31497039
 [6,]
        1 -0.3585686 -0.1091089 0.5217492 -0.5669467 0.31497039
 [7,]
        1 -0.1195229 -0.4364358 0.2981424 0.3779645 -0.62994079
 [8,]
        1 -0.1195229 -0.4364358 0.2981424 0.3779645 -0.62994079
 [9,]
        [10,]
          0.1195229 -0.4364358 -0.2981424 0.3779645 0.62994079
[11,]
        1 0.1195229 -0.4364358 -0.2981424 0.3779645 0.62994079
[12,]
        1 0.1195229 -0.4364358 -0.2981424 0.3779645 0.62994079
[13,]
        1 0.3585686 -0.1091089 -0.5217492 -0.5669467 -0.31497039
[14,]
        1 0.3585686 -0.1091089 -0.5217492 -0.5669467 -0.31497039
        1 0.3585686 -0.1091089 -0.5217492 -0.5669467 -0.31497039
[15,]
[16,]
        1 0.5976143 0.5455447 0.3726780 0.1889822 0.06299408
[17,]
        1 \quad 0.5976143 \quad 0.5455447 \quad 0.3726780 \quad 0.1889822 \quad 0.06299408
[18,]
        1 0.5976143 0.5455447 0.3726780 0.1889822 0.06299408
> probepos <- getProbePosition(leafdevFit)</pre>
> length(probepos)
[1] 753664
> head(probepos)
[1] 50 82 113 177 209 241
> effects <- getEff(leafdevInfCompare)</pre>
> dim(effects)
[1]
       16 753664
> effects[1:8,1:8]
              [,1]
                           [,2]
                                         [,3]
[1,] 2.6268519034 2.6268519034 3.0971474646 3.0971474646
[2,] -0.0340036871 -0.0340036871 -0.0340036871 -0.0340036871
[3,] 0.0050326662 0.0050326662 0.0050326662 0.0050326662
[4,] 0.0390363534 0.0390363534 0.0390363534 0.0390363534
[5,] -0.0338712443 -0.0338712443 -0.0338712443 -0.0338712443
[6,] 0.0001324428 0.0001324428 0.0001324428 0.0001324428
[7,] -0.0389039105 -0.0389039105 -0.0389039105 -0.0389039105
[8,] -0.0386280279 -0.0386280279 -0.0386280279 -0.0386280279
             [,5]
                           [,6]
                                         [,7]
                                                       [,8]
[1,] 3.6378117746 3.6378117746 2.7830456751 2.7830456751
[2,] -0.0340036871 -0.0340036871 -0.0340036871 -0.0340036871
[3,] 0.0050326662 0.0050326662 0.0050326662 0.0050326662
[4,] 0.0390363534 0.0390363534 0.0390363534 0.0390363534
[5,] -0.0338712443 -0.0338712443 -0.0338712443 -0.0338712443
[6,] 0.0001324428 0.0001324428 0.0001324428 0.0001324428
[7,] -0.0389039105 -0.0389039105 -0.0389039105 -0.0389039105
[8,] -0.0386280279 -0.0386280279 -0.0386280279 -0.0386280279
> fdrs <- getFDR(leafdevInfCompare)</pre>
> dim(fdrs)
[1]
       16 753664
```

#### > fdrs[1:8,1:8]

```
[,1]
         [,2]
               [,3]
                     [,4]
                           [,5]
[1,] 0.9346885 0.9346885 0.001058567 0.001058567 1.05712e-15
[,6]
          [,7]
               [,8]
[1,] 1.05712e-15 0.4957921 0.4957921
[2,] 1.00000e+00 1.0000000 1.0000000
[3,] 1.00000e+00 1.0000000 1.0000000
[4,] 1.00000e+00 1.0000000 1.0000000
[5,] 1.00000e+00 1.0000000 1.0000000
[6,] 1.00000e+00 1.0000000 1.0000000
[7,] 1.00000e+00 1.0000000 1.0000000
[8,] 1.00000e+00 1.0000000 1.0000000
```

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