

Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments

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June 20, 2018

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1 Overview

Trendy is an R package for analyzing high throughput expression data (e.g. RNA-seq or microarray) with ordered conditions (e.g. time-course, spatial-course).

For each gene (or other features), Trendy fits a set of segmented (or breakpoint) regression models. The optimal model is chosen as the one with the lowest BIC. Each breakpoint represents a significant changes in the gene's expression across the time-course.

The top dynamic genes are then identified as those that are well profiled by their gene-specific segmented regression model. Trendy also implements functions to visualize the dynamic genes and their trends, to order dynamic genes by their trends, and to compute breakpoint distribution at different time-points (e.g. detect time-points with a large number of expression changes).

1.1 The model

To illustrate Trendy, here we use time-course gene expression data as an example. Although, Trendy may also be applied to other types of features (e.g. isoform or exon expression) and/or other types of experiments with ordered conditions (e.g. spatial course).

Denote the normalized gene expression of gene g and sample/time t as $Y_{g,t}$. Denote the total number of genes as G and the total number of samples/times as N . For each gene, Trendy fits segmented regression models with varying numbers of breakpoints from 1 to K . K defaults to 3 but can also be specified by the user. The [segmented](#) R package is used to fit the segmented regression models.

For a given gene, among the models with varying k , Trendy selects the optimal number of breakpoints for this gene by comparing the BIC for each model.

To avoid overfitting, the optimal number of breakpoints will be set as $\tilde{k}_g = \tilde{k}_g - 1$ if at least one segment has less than c_{num} samples. The threshold c_{num} can be specified by the user; the default is 5.

Trendy reports the following for the optimal model:

- Gene specific adjusted R^2 (penalized for the chosen value of k)
- Segment slopes
- Segment trends (and associated p-values)
- Breakpoint estimates

Among all genes, the top dynamic genes are defined as those whose optimal model has a high adjusted R^2 .

To compute the breakpoint distribution over the time-course, Trendy calculates the number of breakpoints for each time-point across all the genes. The time-points with high D_t can be investigated further as possibly being those with global expression changes.

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Trendy also summarizes the fitted trend or expression pattern of top genes. For samples between the i^{th} and $i+1^{th}$ breakpoint for a given gene, if the t-statistic of the segment slope (slope and standard errors are estimated by the segmented package) has p-value greater than c_{pval} , the trend of this segment will be defined as no change. Otherwise the trend of this segment will be defined as up/down based on the slope coefficient. The default value of c_{pval} is 0.1, but may also be specified by the user.

2 Installation

2.1 Install via Bioconductor

The Trendy package can be installed from Bioconductor if you have R version *geq3.5*.

```
source("https://bioconductor.org/biocLite.R")
biocLite("Trendy")
```

2.2 Install via GitHub

The Trendy package can also be installed using functions in the devtools package.

If you have R version *geq3.5*:

```
install.packages("devtools")
library(devtools)
install_github("rhondabacher/Trendy")
```

For all other R versions, use:

```
install.packages("devtools")
library(devtools)
install_github("rhondabacher/Trendy", ref="devel")
```

2.3 Install locally

Trendy may also be installed locally.

Download the Trendy package from: <https://github.com/rhonda/Trendy>

2.4 Load the package

To load the Trendy package:

```
library(Trendy)
```

3 Analysis

3.1 Input

3.1.1 Normalized Data

The input data should be a $G \times b \times N$ matrix containing the expression values for each gene and each sample, where G is the number of genes and N is the number of samples. The samples should be sorted following the time course order.

The object `trendyExampleData` is a simulated data matrix containing 50 rows of genes and 40 columns of samples.

```
data("trendyExampleData")
str(trendyExampleData)

## num [1:50, 1:40] 240 199 198 239 202 ...
## - attr(*, "dimnames")=List of 2
## ..$ : chr [1:50] "g1" "g2" "g3" "g4" ...
## ..$ : chr [1:40] "s1" "s2" "s3" "s4" ...
```

These values should be expression data after normalization across samples. For example, for RNA-seq data, the raw counts may be normalized using `MedianNorm` (Median Normalization from Anders and Huber (2010)) via the `GetNormalizedMat` function in [EBSeq](#):

```
library(EBSeq)
Sizes <- MedianNorm(trendyExampleData)
normalizedData <- GetNormalizedMat(trendyExampleData, Sizes)
```

More details can be found in the [EBSeq](#) vignette:

http://www.bioconductor.org/packages/devel/bioc/vignettes/EBSeq/inst/doc/EBSeq_Vignette.pdf

If you are working with microarray expression data, an extensive overview for normalization can be found in the vignette of the [affy](#) package:

<https://www.bioconductor.org/packages/release/bioc/html/affy.html>

3.1.2 Time Vector

The time vector is important to specify as it contains the relative timing of each sample. Trendy can handle a number of situations regarding replicates and spacing of time-points. Below are a few examples on how to specify the time vector for a variety of situations:

Suppose all 40 samples are equally spaced time-points:

```
time.vector <- 1:40
time.vector

## [1] 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
## [25] 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
```

Suppose all there are 20 equally spaced time points, each with 2 replicates:

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```
time.vector <- rep(1:20, each = 2)
time.vector

## [1] 1 1 2 2 3 3 4 4 5 5 6 6 7 7 8 8 9 9 10 10 11 11 12 12
## [25] 13 13 14 14 15 15 16 16 17 17 18 18 19 19 20 20
```

Suppose all there are 18 unequally spaced time points, most times have 2 replicates but a few times have 3:

```
time.vector <- c(rep(1, 3), rep(2:9, each = 2), rep(10:11, 3), rep(12:17, each=2), rep(18, 3))
time.vector

## [1] 1 1 1 2 2 3 3 4 4 5 5 6 6 7 7 8 8 9 9 10 11 10 11 10
## [25] 11 12 12 13 13 14 14 15 15 16 16 17 17 18 18 18

table(time.vector)

## time.vector
## 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
## 3 2 2 2 2 2 2 2 2 3 3 2 2 2 2 2 2 3
```

This specification must correspond exactly to the order of samples (columns) in the normalized data matrix described in the previous section.

3.2 Run Trendy

The `trendy` function will fit multiple segmented regressions models for each gene (via the `segmented` R package) and select the the optimal model. For this example, we will only consider a maximum of two breakpoints for each gene.

```
time.vector <- 1:40
res <- trendy(Data = trendyExampleData, tVectIn = time.vector, maxK = 2)
res <- results(res)
res.top <- topTrendy(res)
# default adjusted R square cutoff is 0.5
res.top$AdjustedR2

##      g3      g1      g28      g20      g15      g4      g2
## 0.9787382 0.9775005 0.9751380 0.9739715 0.9729747 0.9711890 0.9710139
##      g10      g23      g14      g8      g5      g24      g17
## 0.9705118 0.9701402 0.9694164 0.9691341 0.9689555 0.9656732 0.9652141
##      g9      g12      g29      g16      g22      g18      g6
## 0.9649424 0.9644343 0.9632348 0.9630272 0.9627092 0.9626837 0.9619387
##      g25      g11      g30      g27      g26      g19      g7
## 0.9611528 0.9600736 0.9597989 0.9592516 0.9572072 0.9536619 0.9529077
##      g21      g13
## 0.9528854 0.9470928
```

The `topTrendy` function may be used to extract top dynamic genes. By default, `topTrendy` will extract genes whose adjusted R^2 , \bar{R}^2 , is greater or equal to 0.5. To change this threshold, a user may specify the `adjR2Cut` parameter in the `topTrendy` function. The `topTrendy` function returns the Trendy output with genes sorted decreasingly by \bar{R}^2 .

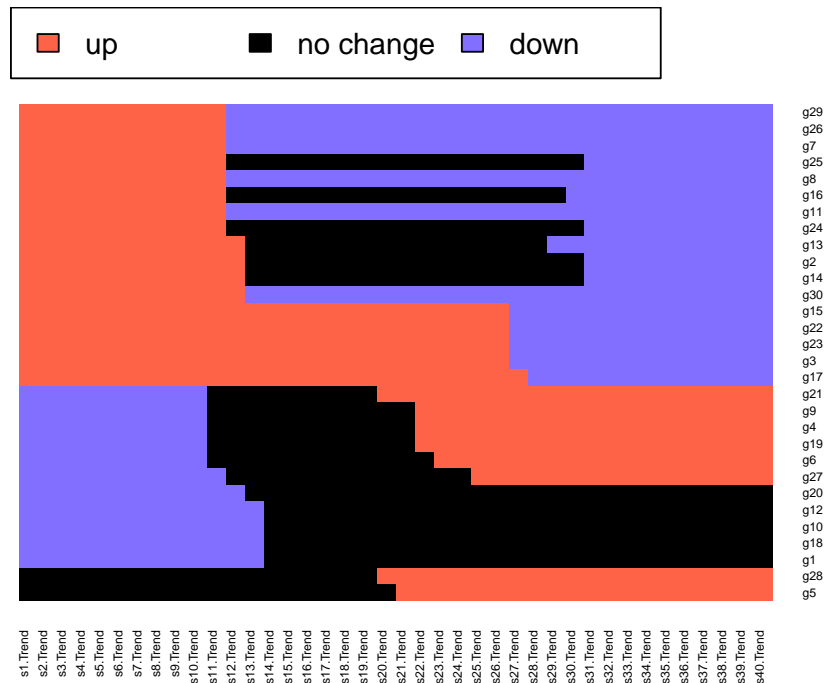
By default the `trendy` function only considers genes whose mean expression is greater than 10. To use another threshold, the user may specify the parameter `meanCut`.

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3.3 Visualize trends of the top dynamic genes

`res.top$Trend` contains the trend specification of the top genes. The function `trendHeatmap` can be used to display these trends. First, the `trendHeatmap` function classifies the top dynamic genes into three groups: those that start with 'up', start with 'down' and start with 'no change'. Within each group, genes are sorted by the position of the first breakpoint.

```
res.trend <- trendHeatmap(res.top)
```



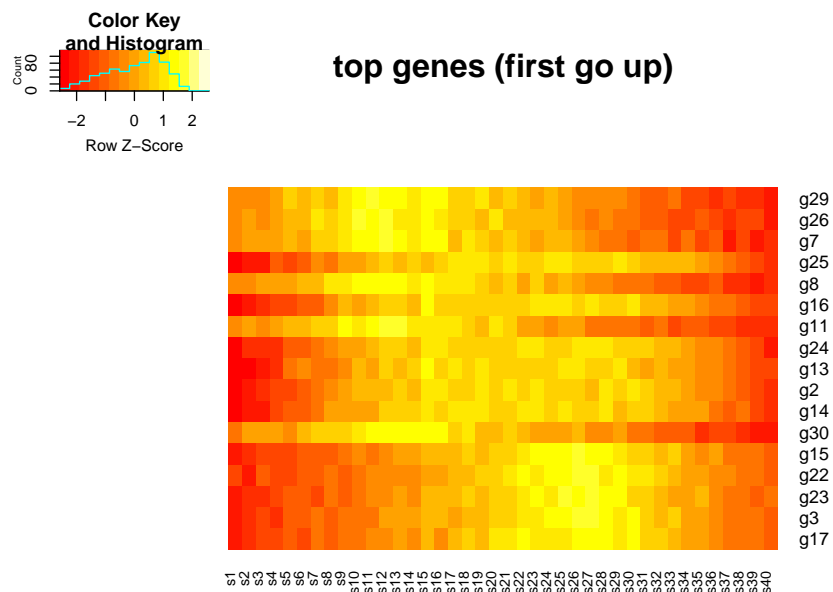
```
str(res.trend)

## List of 3
## $ firstup      : Named num [1:17] 11.4 11.5 11.6 11.6 11.6 ...
## .. attr(*, "names")= chr [1:17] "g29" "g26" "g7" "g25" ...
## $ firstdown    : Named num [1:11] 10.7 10.9 10.9 10.9 11 ...
## .. attr(*, "names")= chr [1:11] "g21" "g9" "g4" "g19" ...
## $ firstnochange: Named num [1:2] 19 20.4
## .. attr(*, "names")= chr [1:2] "g28" "g5"
```

To generate an expression heatmap of the first group of genes (first go 'up'):

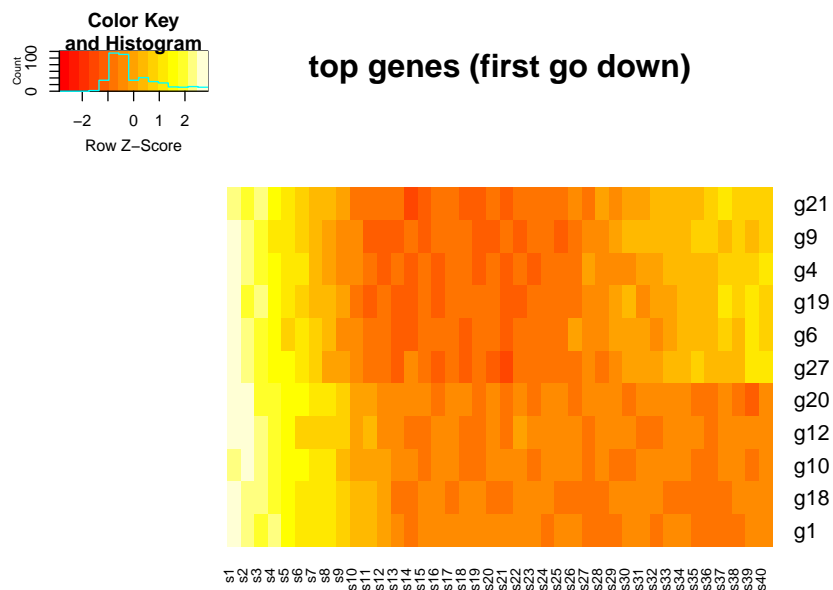
```
library(gplots)
heatmap.2(trendyExampleData[names(res.trend$firstup),],
  trace="none", Rowv=FALSE, Colv=FALSE,
  scale="row", main="top genes (first go up)")
```

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Similarly, to generate an expression heatmap of the second group of genes (first go down):

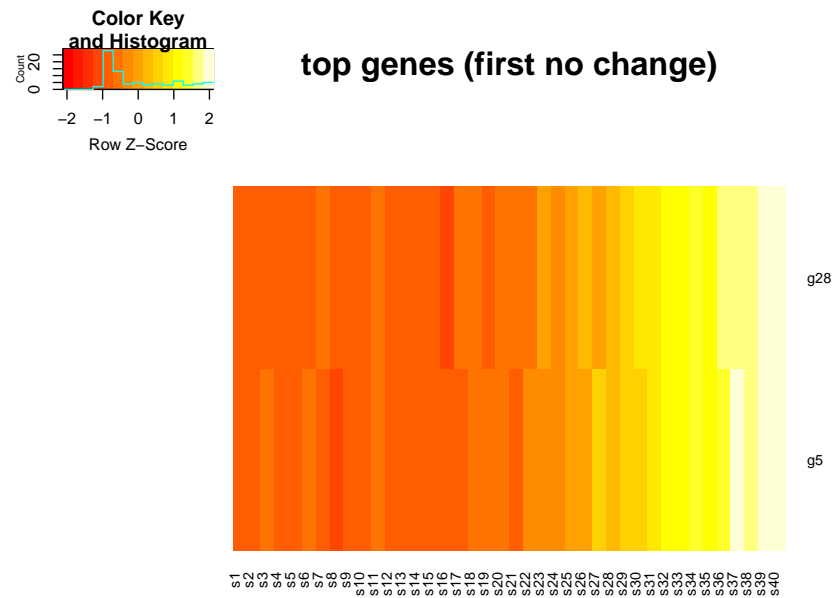
```
heatmap.2(trendyExampleData[names(res.trend$firstdown),],
  trace="none", Rowv=FALSE, Colv=FALSE,
  scale="row", main="top genes (first go down)")
```



To generate an expression heatmap of the second group of genes (first no change):

```
heatmap.2(trendyExampleData[names(res.trend$firstnochange),],
  trace="none", Rowv=FALSE, Colv=FALSE,
  scale="row", main="top genes (first no change)",
  cexRow=.8)
```

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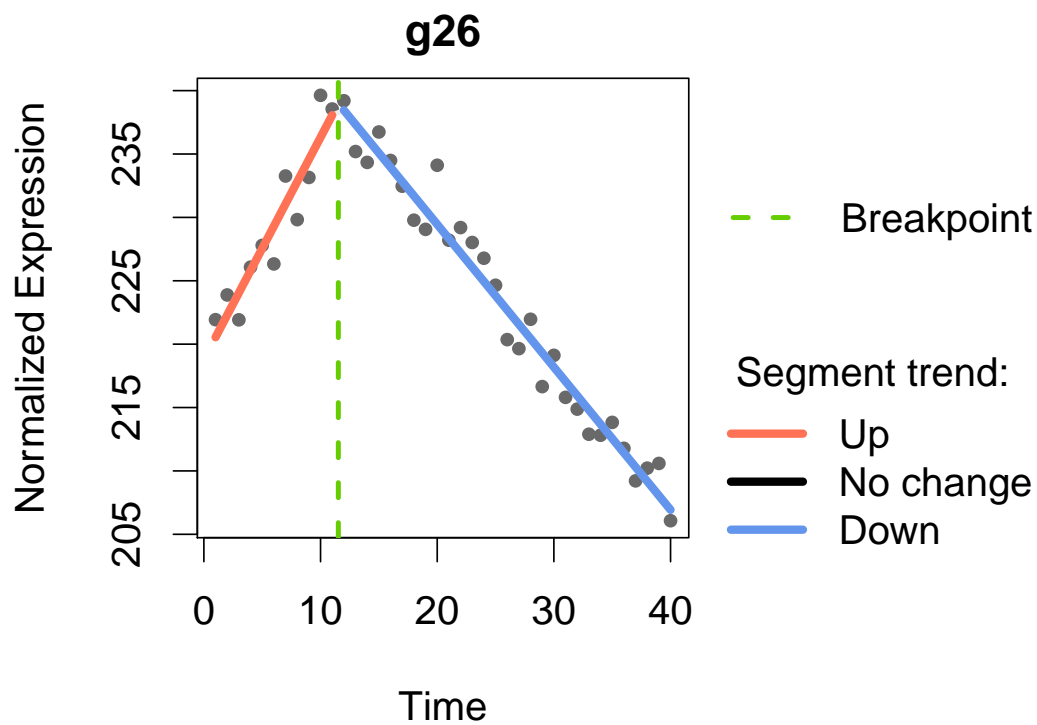
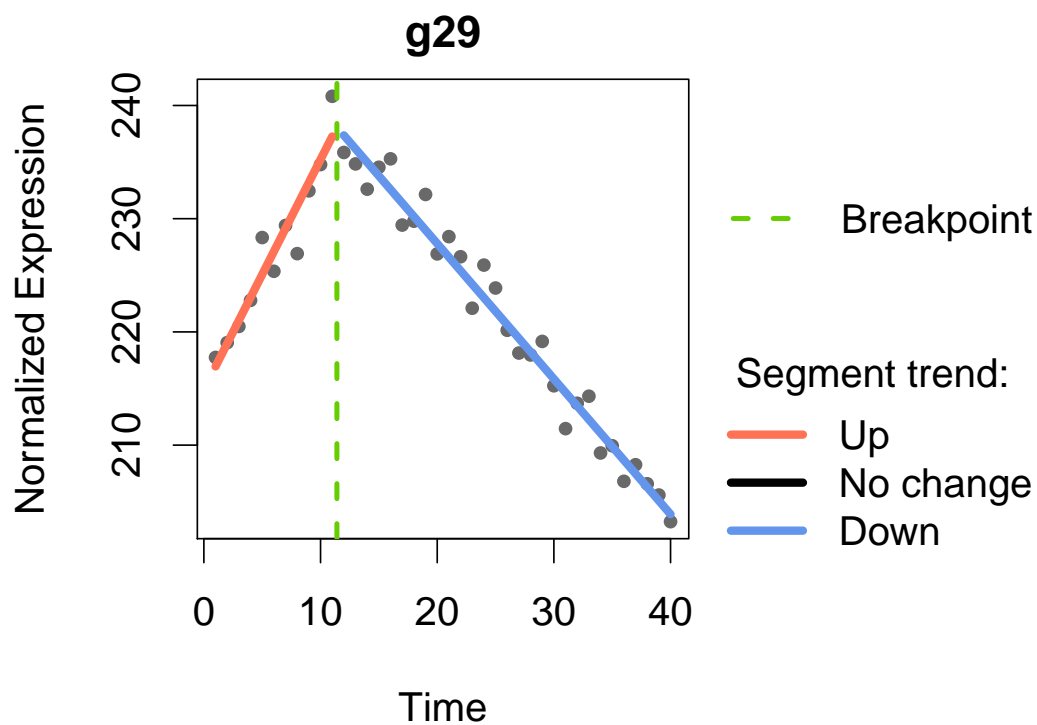
3.4 Visualize individual genes

The `plotFeature` function may be used to plot expression of individual features/genes and the fitted lines.

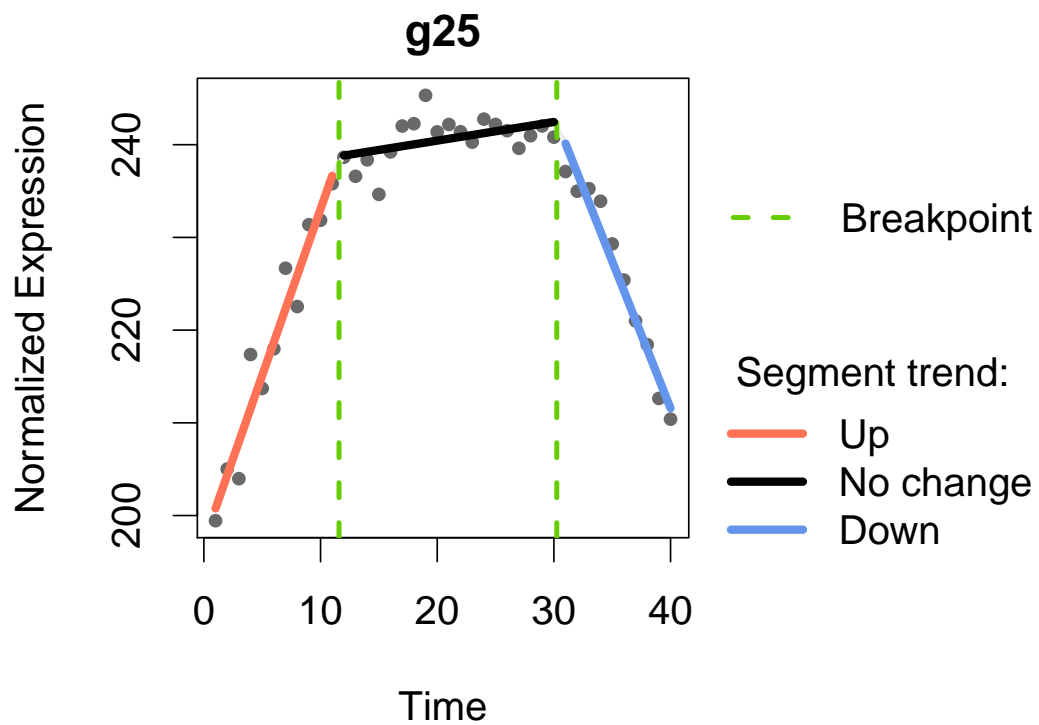
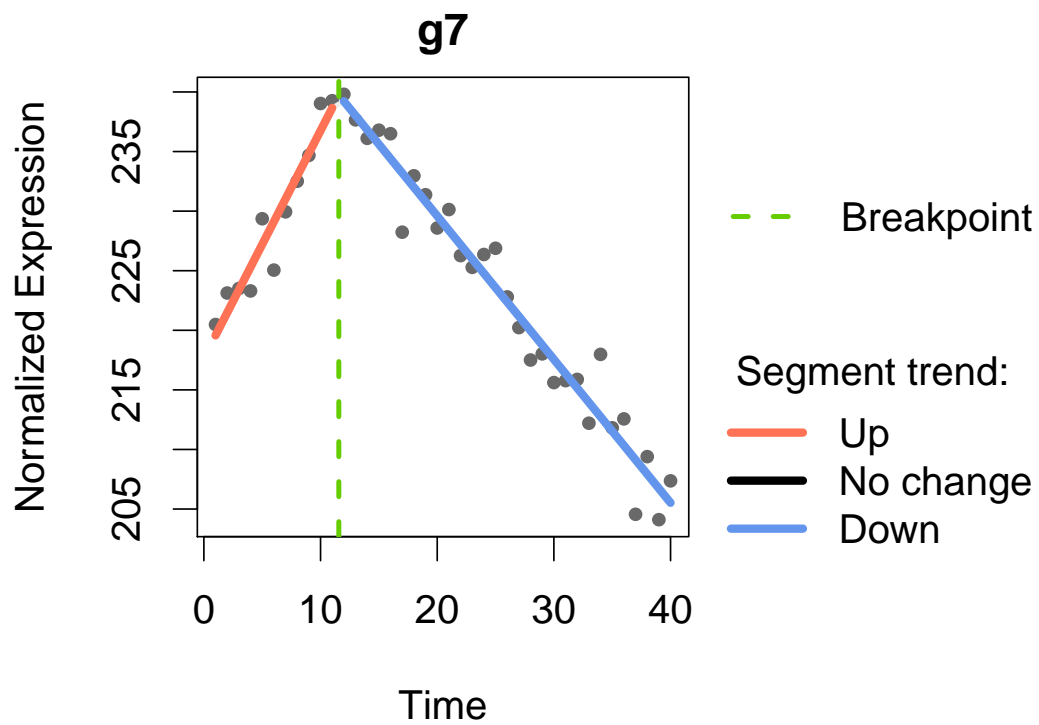
For example, to plot the top six genes in the first group of genes (first go up):

```
par(mfrow=c(3,2))
plot1 <- plotFeature(trendyExampleData,tVectIn = time.vector,
                     featureNames = names(res.trend$firstup)[1:6],
                     trendyOut = res)
```

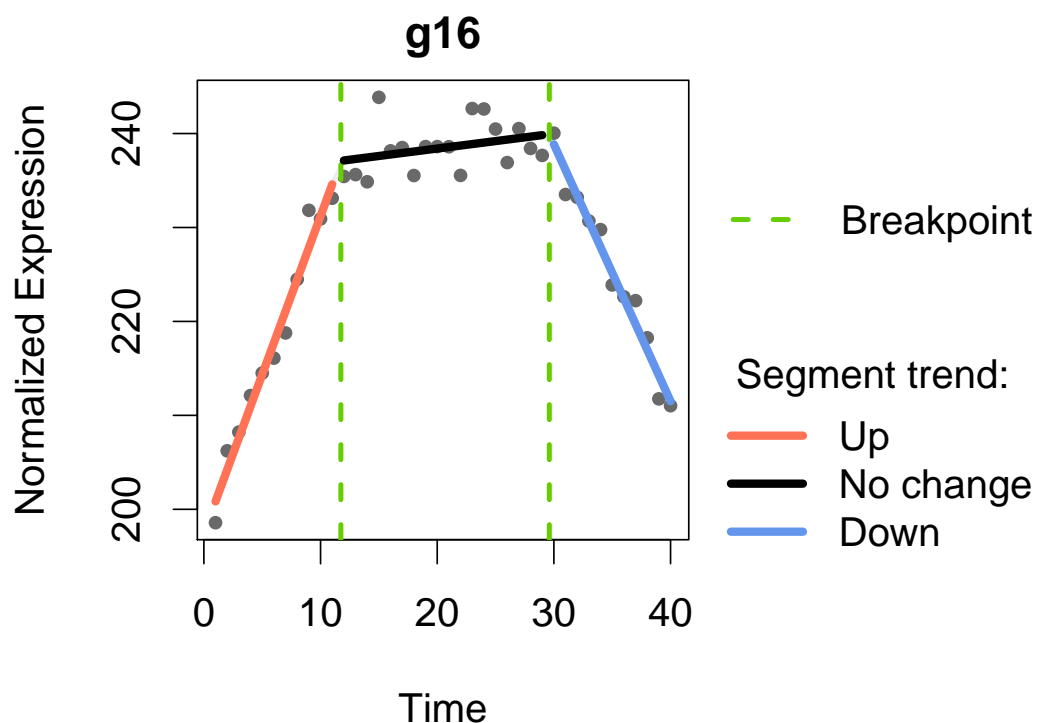
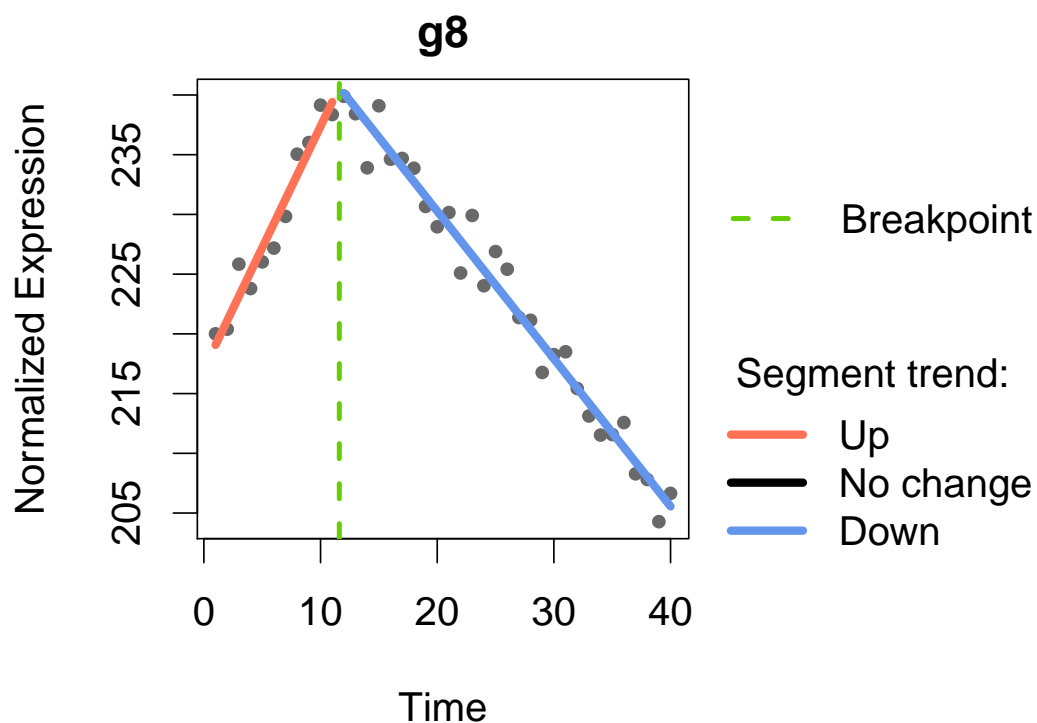

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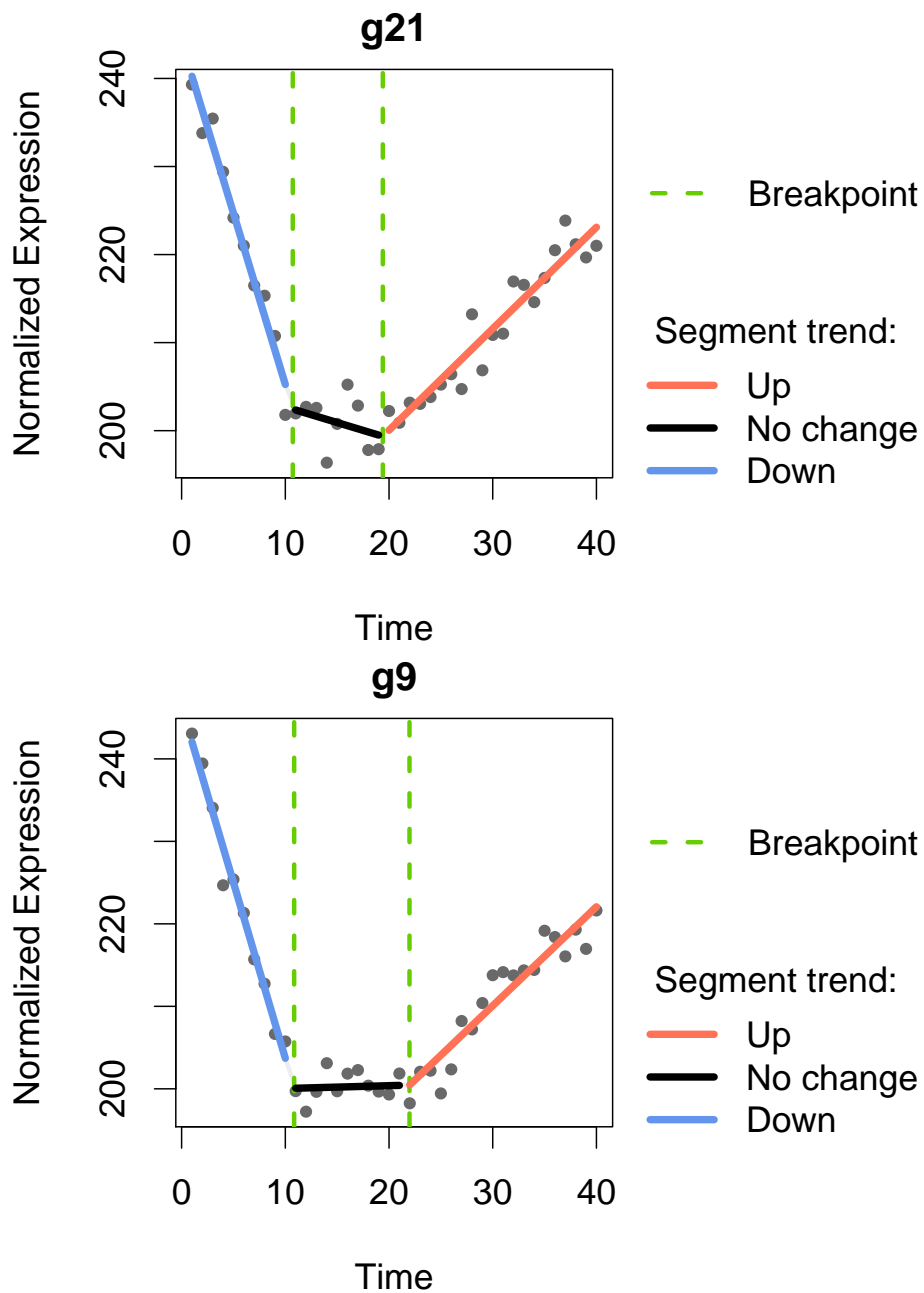


The input of function `plotFeature` requires the expression data and a list of genes of interest. The parameter `trendyOut` are the results from the `trendy` function. If it is not specified, then `plotFeature` will run `trendy` on the genes of interest before plotting. Specifying the output obtained from previous steps will save time by avoiding fitting the models again.

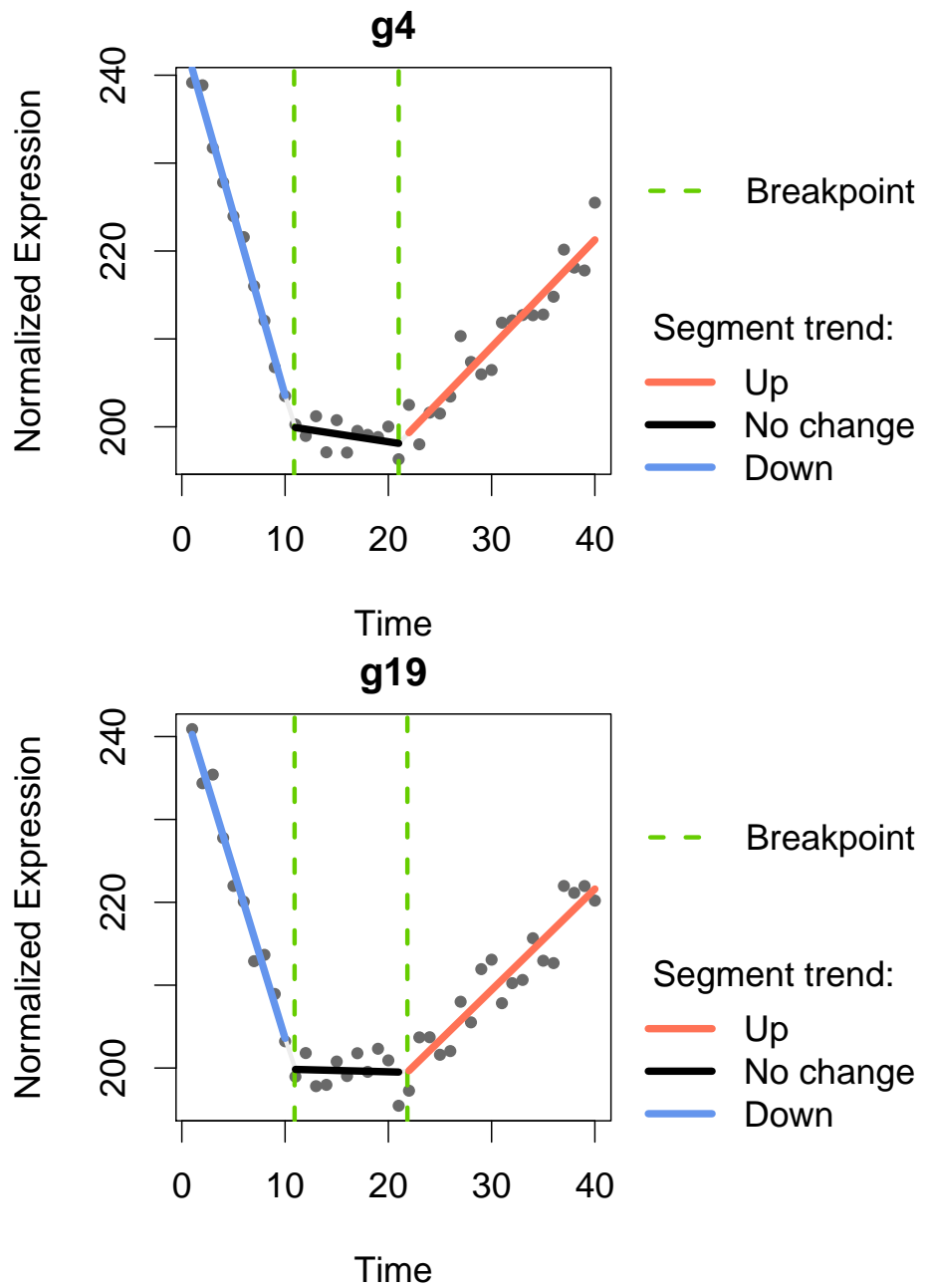
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Similarly, to plot the top six genes in the second group of genes (first go down):

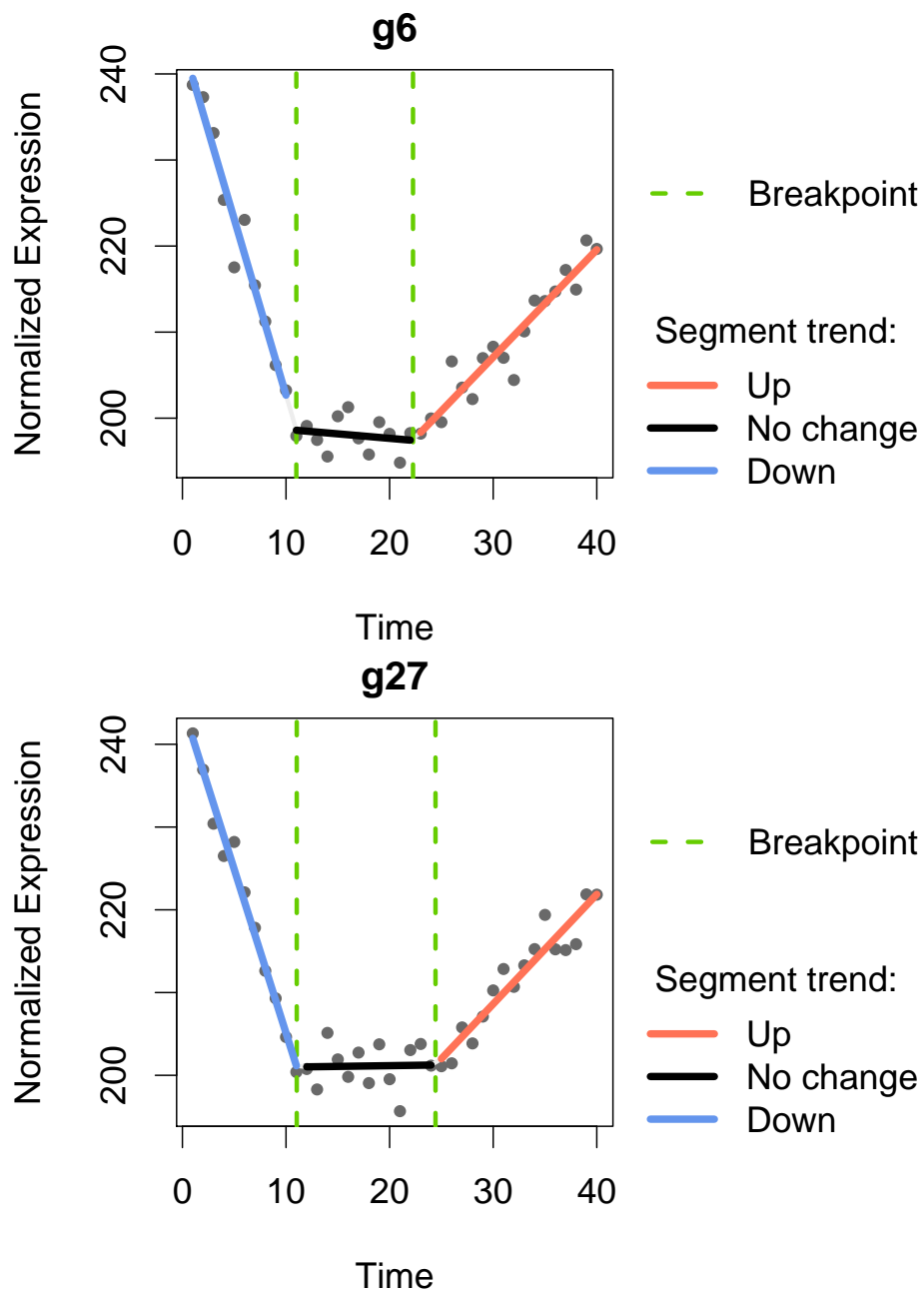
```
par(mfrow=c(3,2))
plot2 <- plotFeature(Data = trendyExampleData, tVectIn = time.vector,
  featureNames = names(res.trend$firstdown)[1:6],
  trendyOut = res)
```



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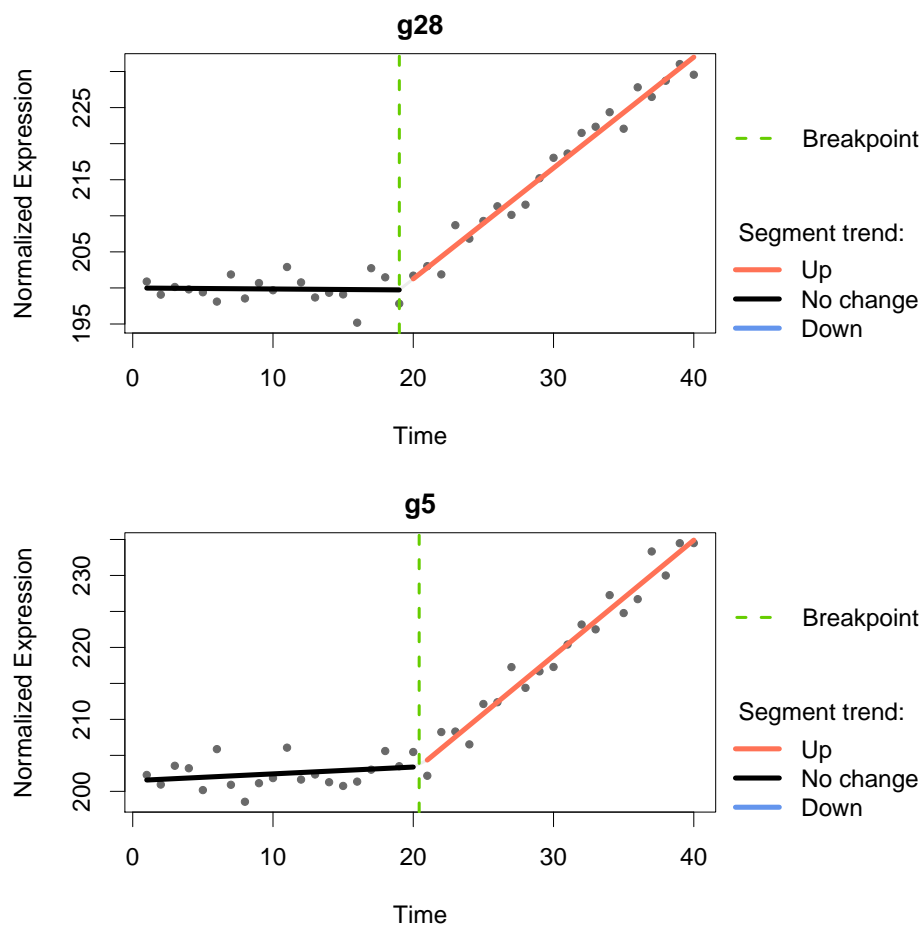
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To plot the two genes in the third group of genes (first no change):

```
par(mfrow=c(1,2))
plot2 <- plotFeature(trendyExampleData,tVectIn = time.vector,
                     featureNames = names(res.trend$firstnochange)[1:2],
                     trendyOut = res)
```

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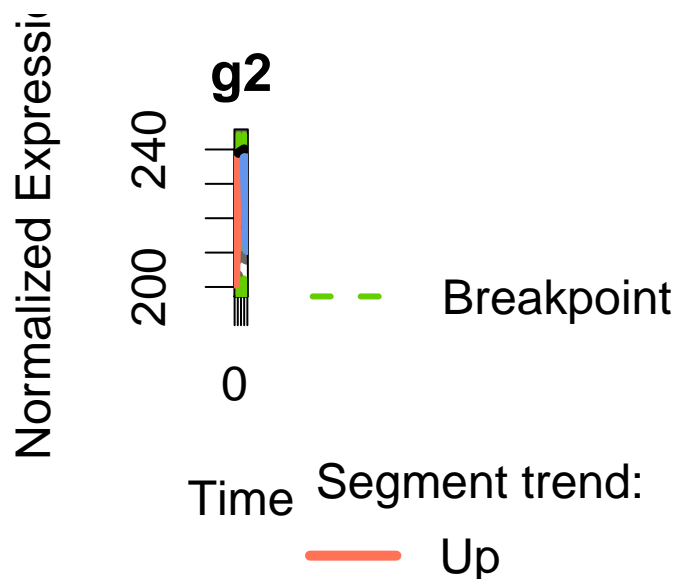


3.5 Gene specific estimates

For a given gene of interest, its estimated parameters can be obtained individually:

```
par(mfrow=c(1,1))
plot2 <- plotFeature(trendyExampleData,tVectIn = time.vector,
                     featureNames = "g2",
                     trendyOut = res)
```

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```
res.top$Breakpoints["g2",] # break points
## Breakpoint1 Breakpoint2
## 12.47356 30.14908

res.top$AdjustedR2["g2"] # adjusted r squared
## g2
## 0.9710139

res.top$Segments["g2",] # fitted slopes of the segments
## NULL

res.top$Segment.Pvalues["g2",] # p value of each the segment
## Segment1.Pvalue Segment2.Pvalue Segment3.Pvalue
## 0.01669823 0.31816176 0.02445599
```

The above printout show that for gene g2 the optimal number of breakpoints is 2. Two estimated breakpoints are around time-points 12 and 30. The fitted slopes for the 3 segments are 3.31, 0.06 and -2.97, which indicate the trend is 'up'-'no change'-'down.'

These estimates can be automatically formatted using the function `formatResults` which can then be saved as a .txt. or .csv file. The output currently includes the estimated slope, p-value, and trend of each segment, the estimated breakpoints, the trend for each sample, and the adjusted R^2 .

```
trendy.summary <- formatResults(res.top)
trendy.summary[1:4,1:8]

## Feature Segment1.Slope Segment2.Slope Segment3.Slope Segment1.Trend
## g3 g3 1.572400 -2.5483000 NA 1
## g1 g1 -3.145400 0.0015484 NA -1
## g28 g28 -0.013815 1.5369000 NA 0
## g20 g20 -3.381400 -0.0824630 NA -1
## Segment2.Trend Segment3.Trend Segment1.Pvalue
## g3 -1 NA 0.009070596
## g1 0 NA 0.013816451
## g28 1 NA 0.440273456
## g20 0 NA 0.015765320
```


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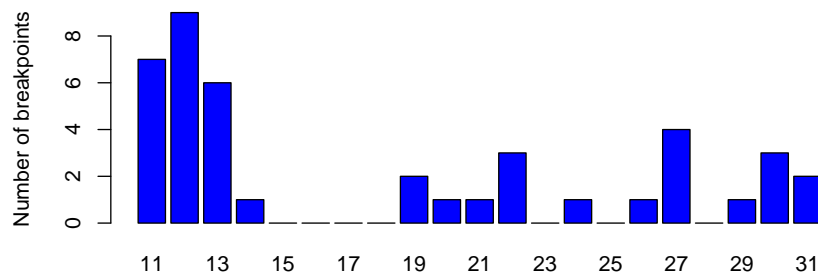
```
# To save:  
# write.table(trendy.summary, file="trendy_summary.txt")
```

The NA indicates that g3 does not have a segment 3 slope since it only has one breakpoint (i.e two segments).

3.6 Breakpoint distribution over the time course

To calculate number of breakpoints for all genes over the time course:

```
res.bp <- breakpointDist(res.top)  
barplot(res.bp, ylab="Number of breakpoints", col="blue")
```



The bar plot indicates that a number of genes have breakpoints around times 12 and 13.

4 More advanced analysis

4.1 Time course with non-uniform sampling

If the samples were collected at different time intervals then it is highly suggested to use the original time (instead of a vector of consecutive numbers). To do so, the user may specify the order/times via the `tVectIn` parameter in the `trendy` function.

For example, suppose for the simulated data, the first 30 samples were collected every hour and the other 10 samples were collected every 5 hours. We may define the time vector as:

```
time.vector <- c(1:30, seq(31, 80, 5))  
names(time.vector) <- colnames(trendyExampleData)  
time.vector  
  
## s1 s2 s3 s4 s5 s6 s7 s8 s9 s10 s11 s12 s13 s14 s15 s16 s17 s18 s19  
## 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19  
## s20 s21 s22 s23 s24 s25 s26 s27 s28 s29 s30 s31 s32 s33 s34 s35 s36 s37 s38  
## 20 21 22 23 24 25 26 27 28 29 30 31 36 41 46 51 56 61 66  
## s39 s40  
## 71 76
```

To run Trendy model using the empirical collecting time instead of sample ID (1-40):

```
res2 <- trendy(Data = trendyExampleData, tVectIn = time.vector, maxK=2)  
res2 <- results(res2)  
res.top2 <- topTrendy(res2)  
res.trend2 <- trendHeatmap(res.top2)
```

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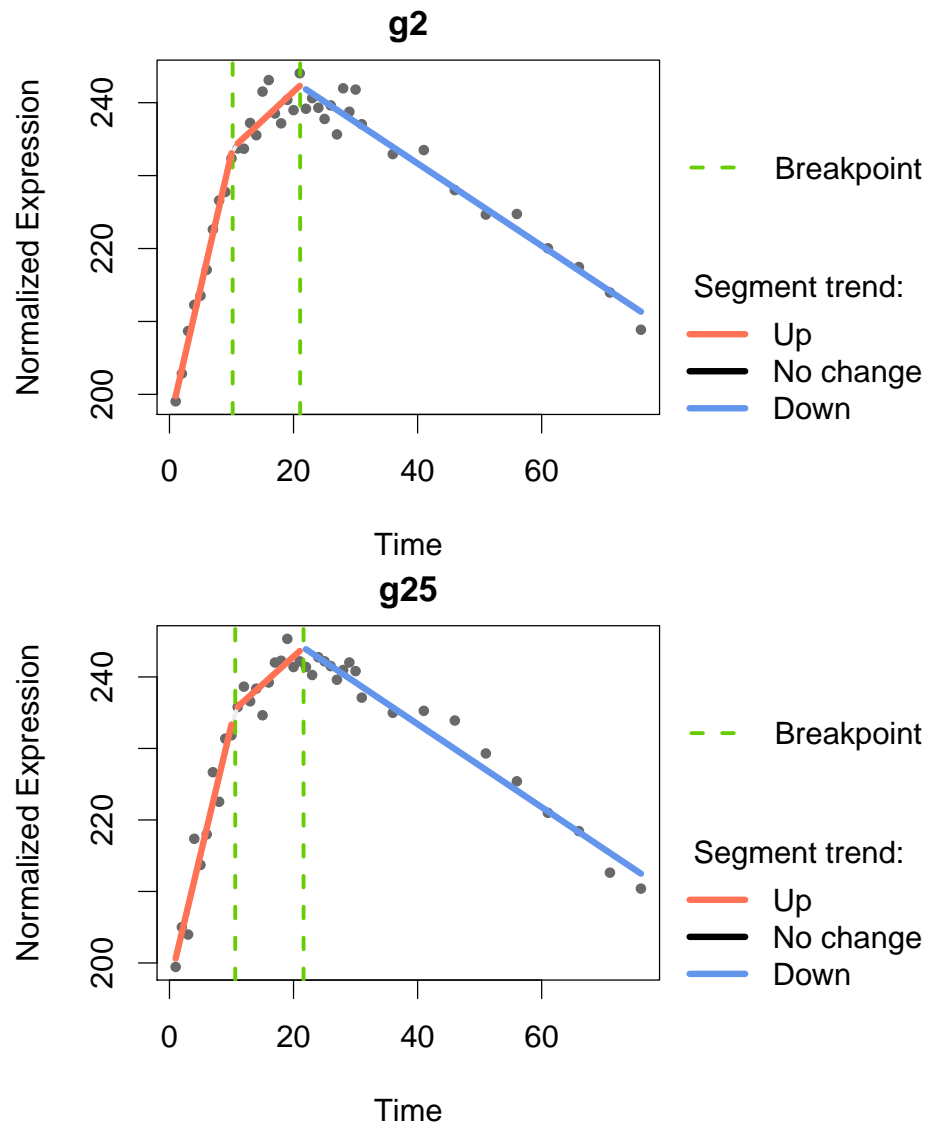
```
str(res.trend2)

## List of 3
## $ firstup      : Named num [1:17] 10.2 10.6 11.4 11.4 11.4 ...
## .. attr(*, "names")= chr [1:17] "g2" "g25" "g29" "g16" ...
## $ firstdown    : Named num [1:11] 11.2 11.4 11.4 11.5 11.7 ...
## .. attr(*, "names")= chr [1:11] "g19" "g4" "g27" "g6" ...
## $ firstnochange: Named num [1:2] 19 19.5
## .. attr(*, "names")= chr [1:2] "g28" "g5"
```

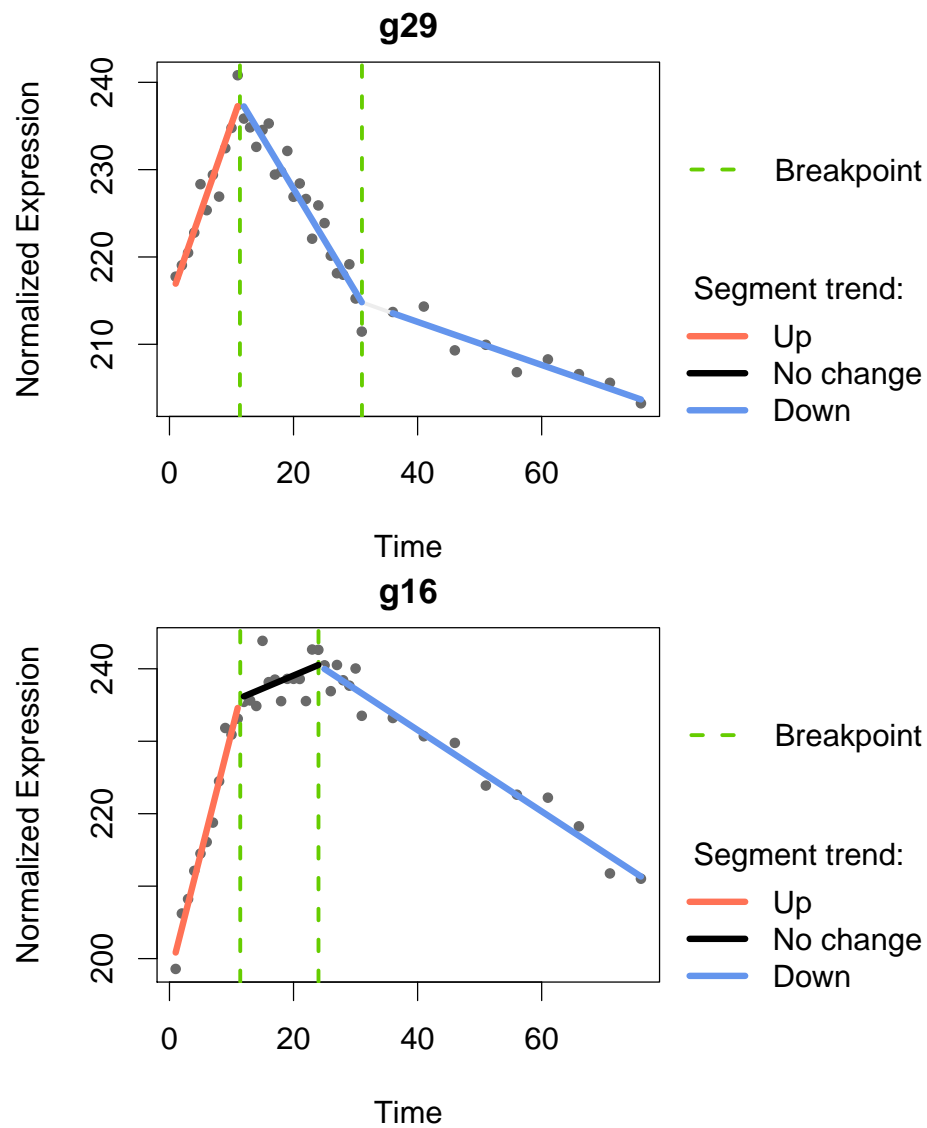
To plot the first six genes that have up-regulated pattern at the beginning of the time course:

```
par(mfrow=c(3,2))
plot1.new <- plotFeature(trendyExampleData, tVectIn=time.vector,
                        featureNames = names(res.trend2$firstup)[1:6],
                        trendyOut = res2)
```

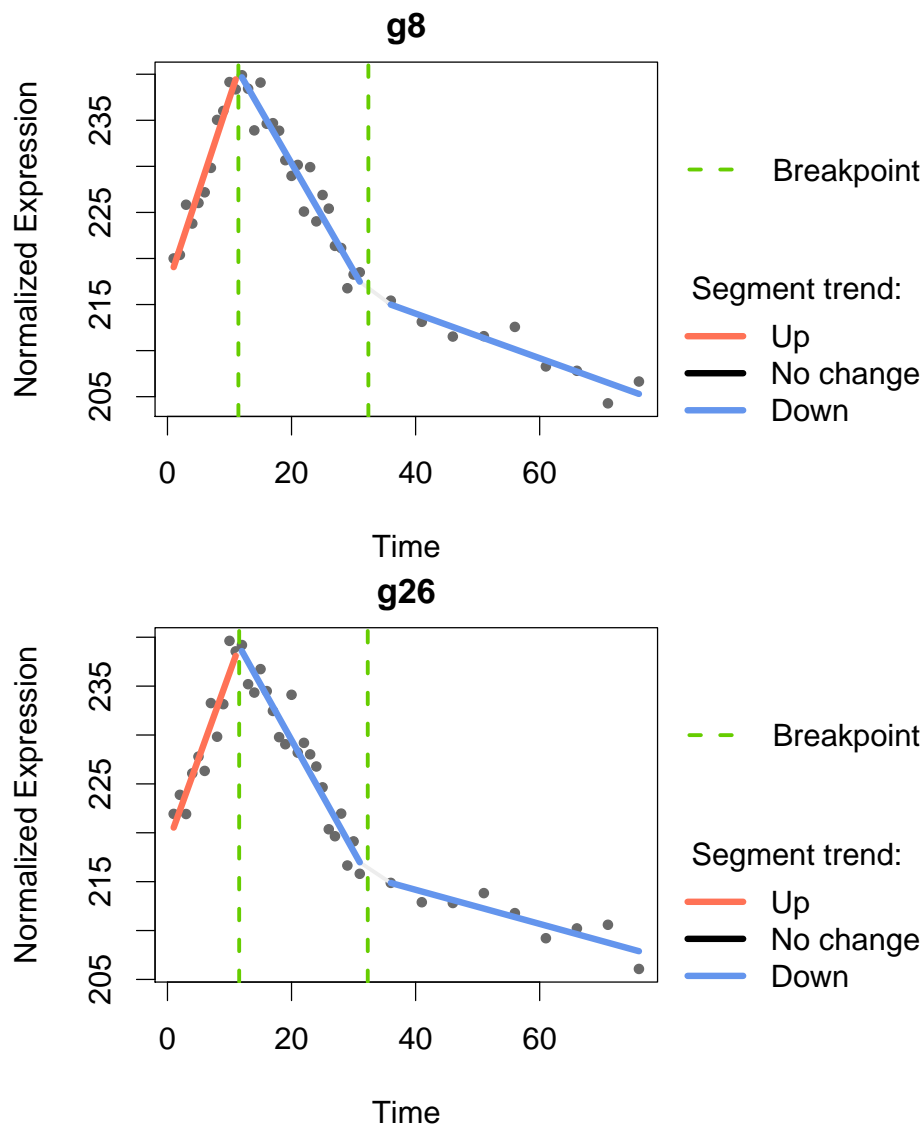
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4.2 Time-course with replicates available

Trendy is able to make use of replicated time-points if available. To do so, the user can specify the replicated directly into the the `tVectIn` parameter in the `trendy` function.

For example, suppose for the example data, 10 time points were observed 4 times each. We may define the time vector as:

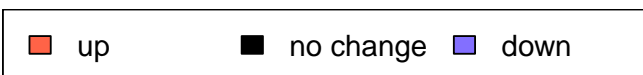
```
t.v <- rep(1:10, each=4)
names(t.v) <- colnames(trendyExampleData)
t.v
```

```
## s1 s2 s3 s4 s5 s6 s7 s8 s9 s10 s11 s12 s13 s14 s15 s16 s17 s18 s19
## 1 1 1 1 2 2 2 2 3 3 3 3 4 4 4 4 5 5 5
## s20 s21 s22 s23 s24 s25 s26 s27 s28 s29 s30 s31 s32 s33 s34 s35 s36 s37 s38
## 5 6 6 6 6 7 7 7 7 8 8 8 8 9 9 9 9 10 10
```

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```
## s39 s40
## 10 10
```

```
res2 <- trendy(Data = trendyExampleData, tVectIn = time.vector, maxK=2)
res2 <- results(res2)
res.top2 <- topTrendy(res2)
res.trend2 <- trendHeatmap(res.top2)
```



```
str(res.trend2)

## List of 3
## $ firstup      : Named num [1:17] 10.2 10.6 11.4 11.4 11.4 ...
## .. attr(*, "names")= chr [1:17] "g2" "g25" "g29" "g16" ...
## $ firstdown    : Named num [1:11] 11.2 11.4 11.4 11.5 11.7 ...
## .. attr(*, "names")= chr [1:11] "g19" "g4" "g27" "g6" ...
## $ firstnochange: Named num [1:2] 19 19.5
## .. attr(*, "names")= chr [1:2] "g28" "g5"
```

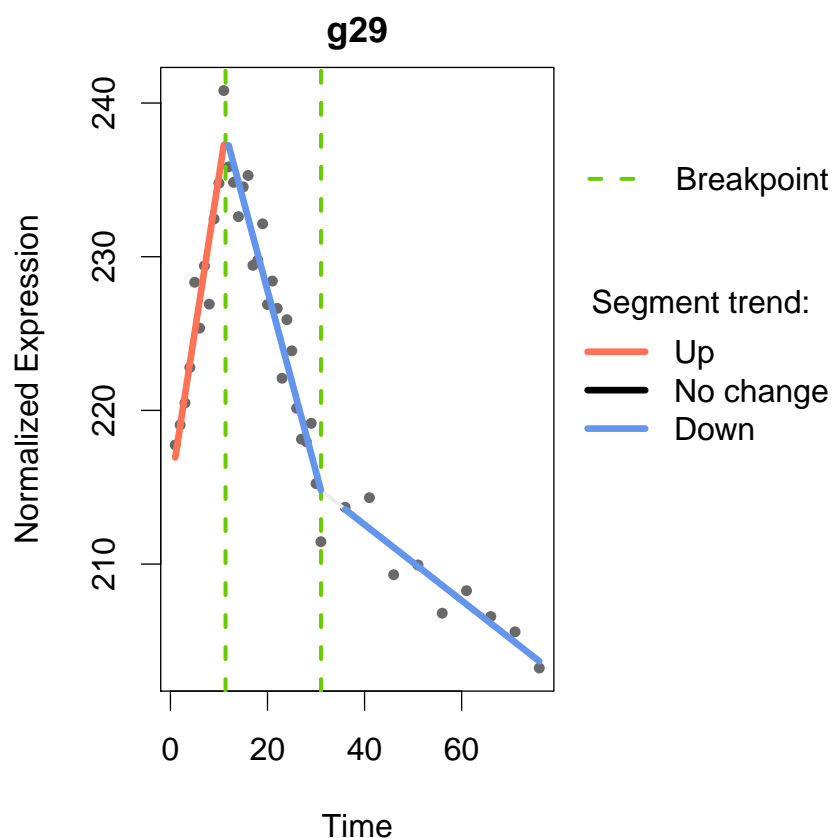
5 Extract genes with certain pattern

Genes that have a peak along the time-course will have fitted trend somewhere as "up-down". Genes that are oscillating may have the fitted trend "up-down". To extract a list of such genes we can use the `extractPattern`:

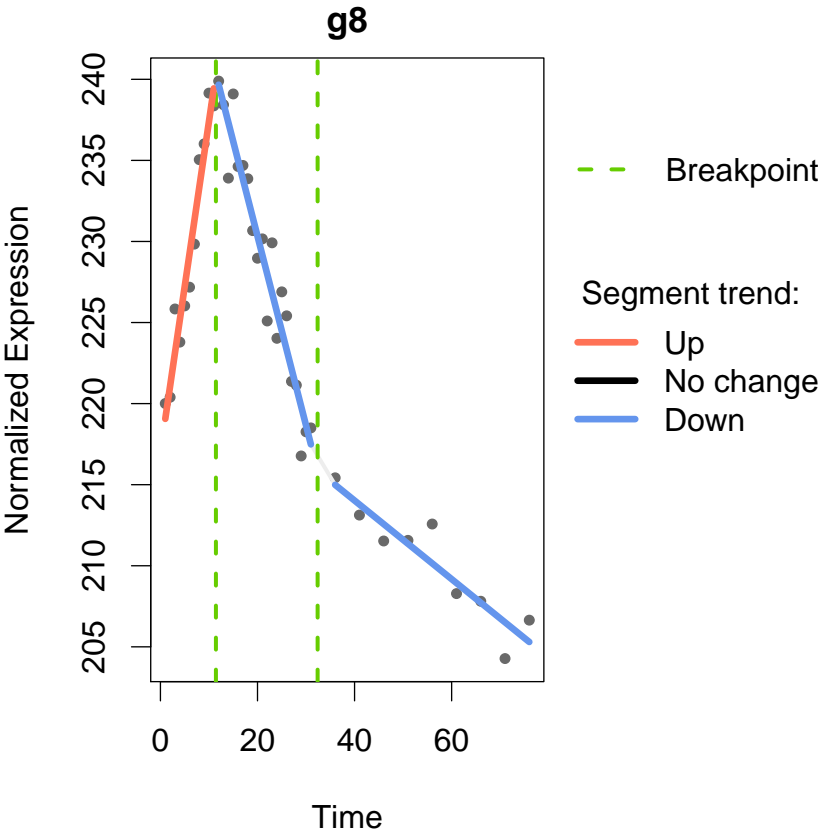
```
# Genes that peak
pat1 <- extractPattern(res2, Pattern = c("up", "down"))
head(pat1)

##      Gene BreakPoint1
## 5    g29    11.35849
## 2     g8    11.43482
## 8    g26    11.56147
## 10   g7     11.65663
## 4    g11    11.99780
## 6    g30    12.38463

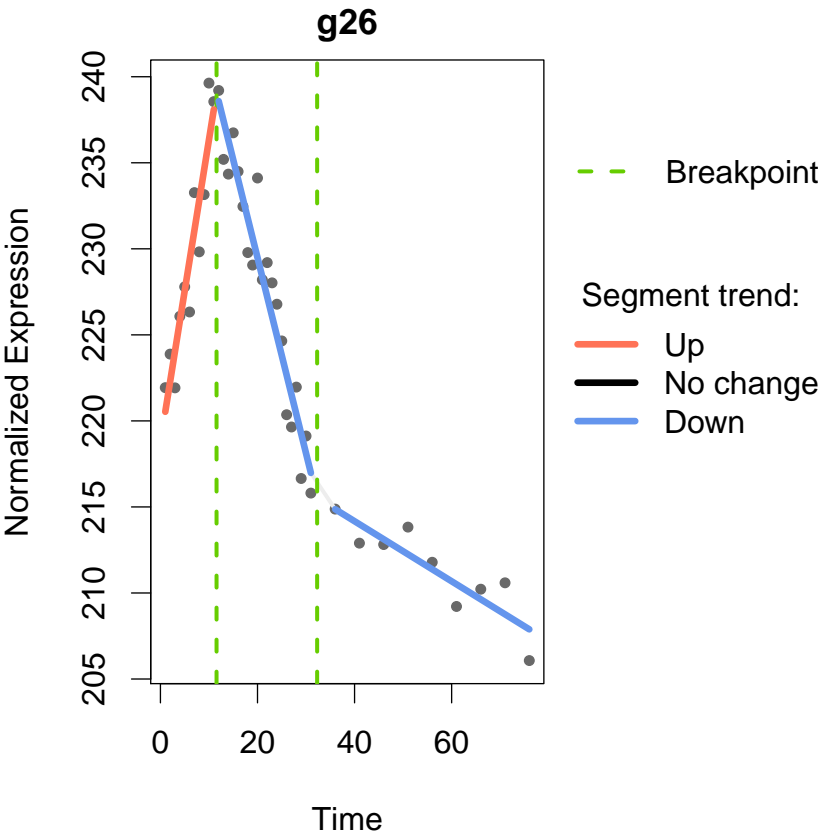
par(mfrow=c(3,2))
plotPat1 <- plotFeature(trendyExampleData, tVectIn=time.vector,
                        featureNames = pat1$Gene[1:6],
                        trendyOut = res2)
```



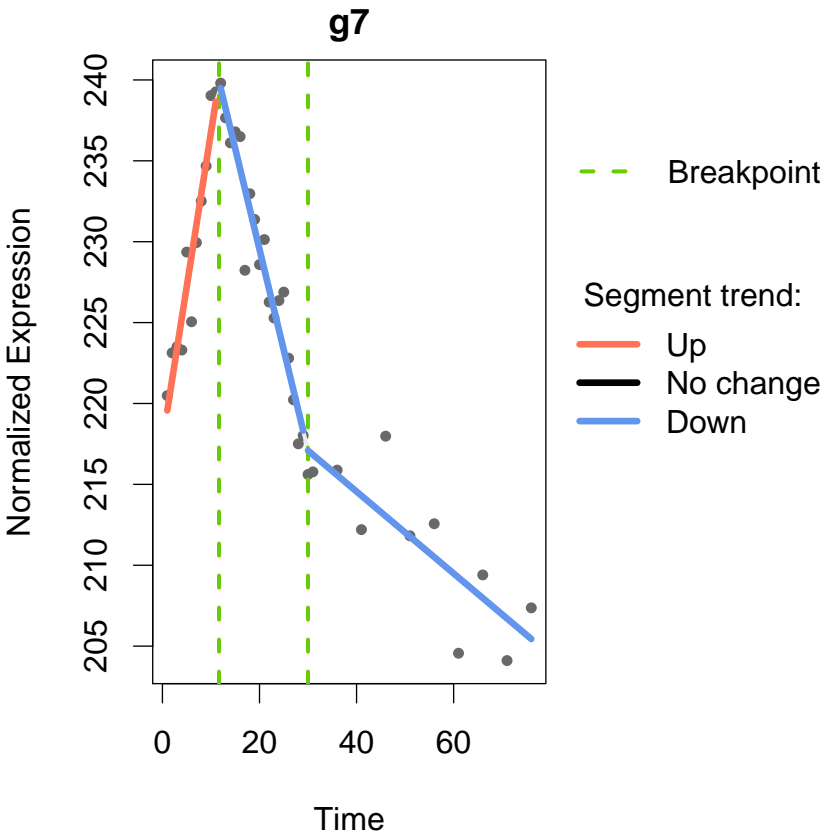
Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



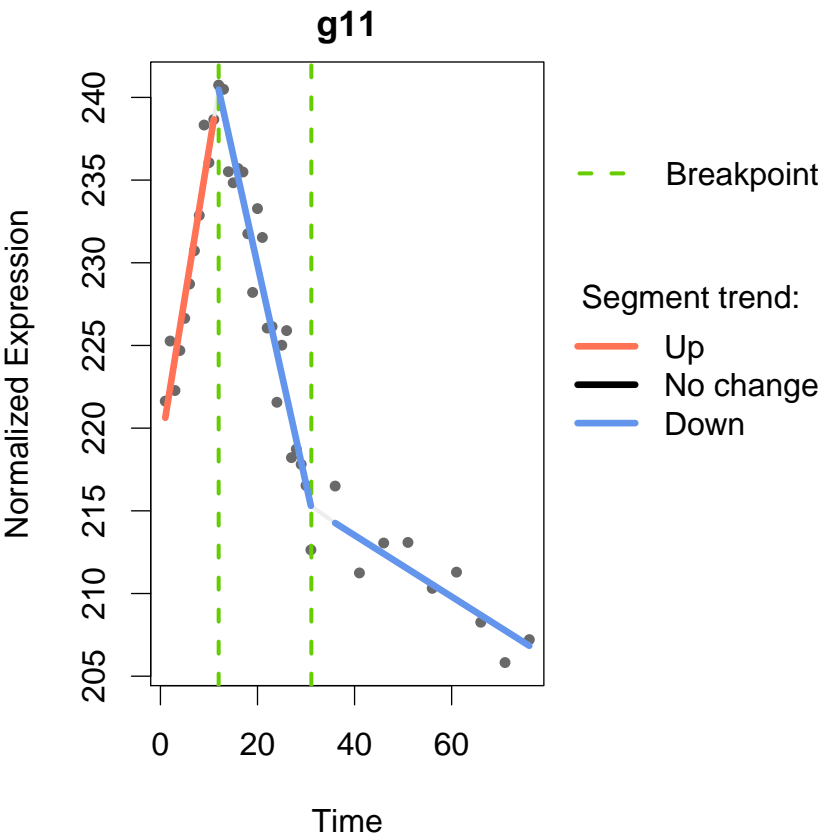
Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



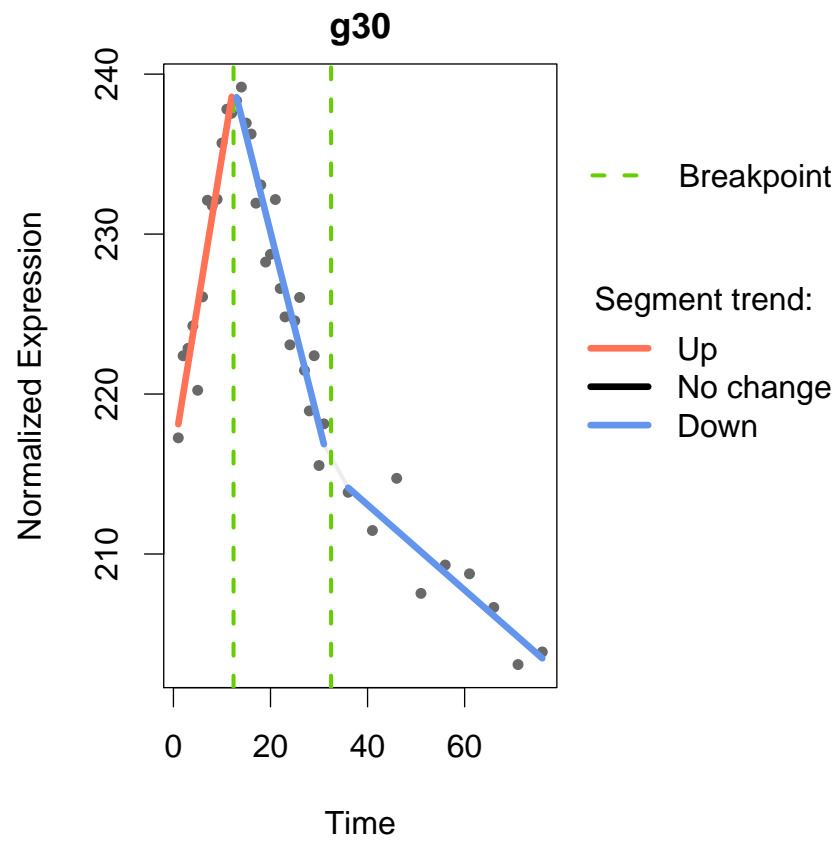
Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments

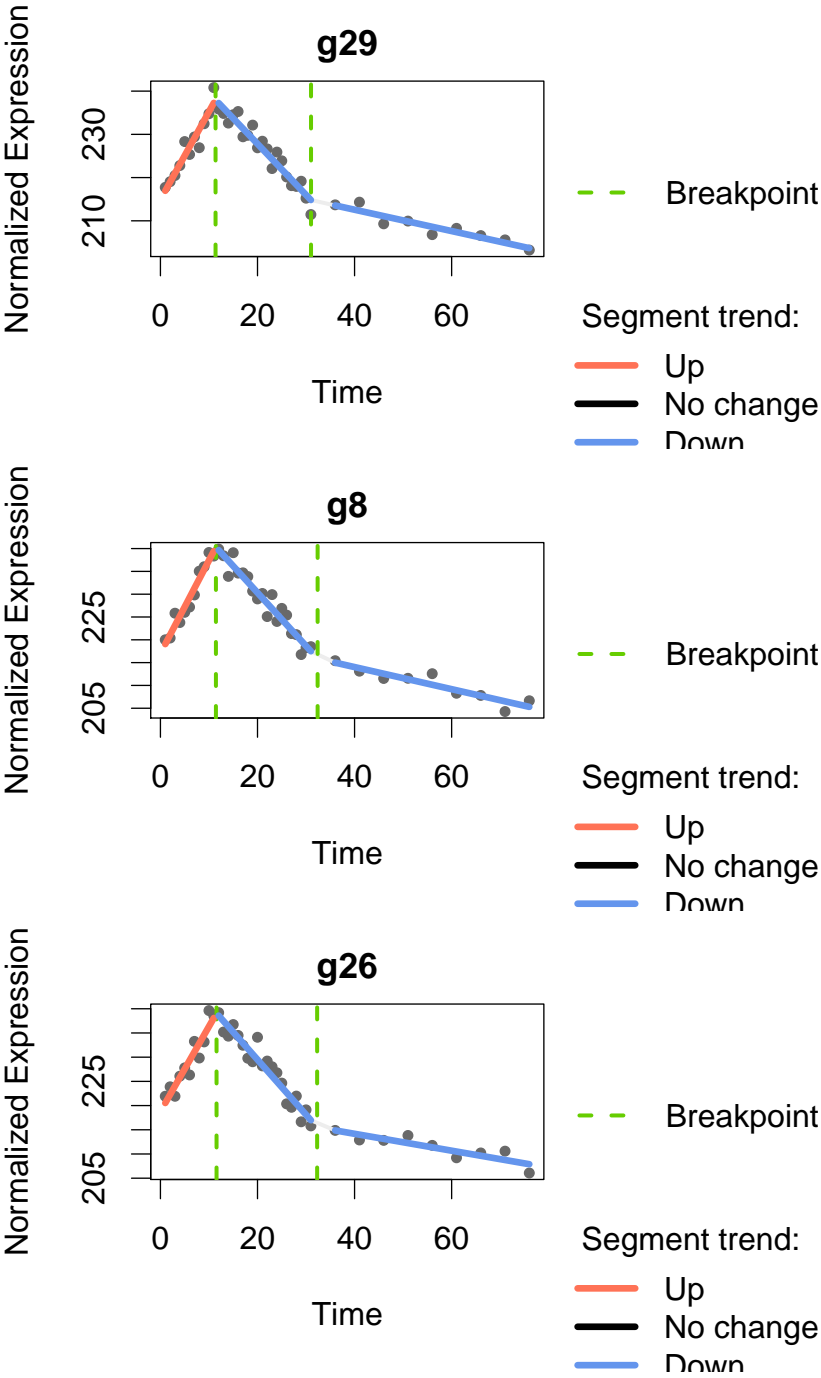


```
# Genes that peak after some time
pat3 <- extractPattern(res2, Pattern = c("up", "down"), Delay = 25)
head(pat3)

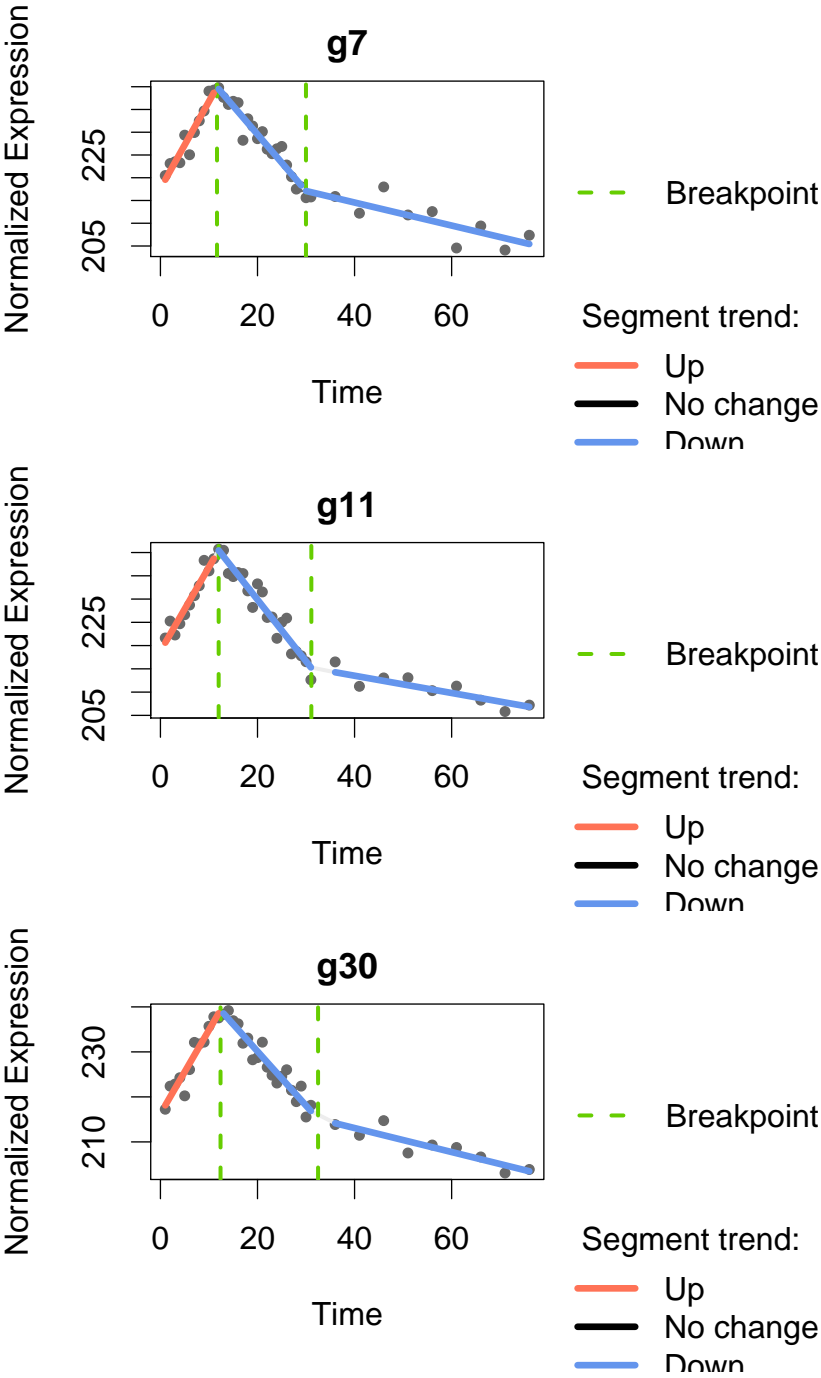
##      Gene BreakPoint1
## 5    g29    11.35849
## 2     g8    11.43482
## 8    g26    11.56147
## 10   g7     11.65663
## 4    g11    11.99780
## 6    g30    12.38463

par(mfrow=c(1,2))
plotPat3 <- plotFeature(trendyExampleData, tVectIn=time.vector,
                        featureNames = pat3$Gene,
                        trendyOut = res2)
```

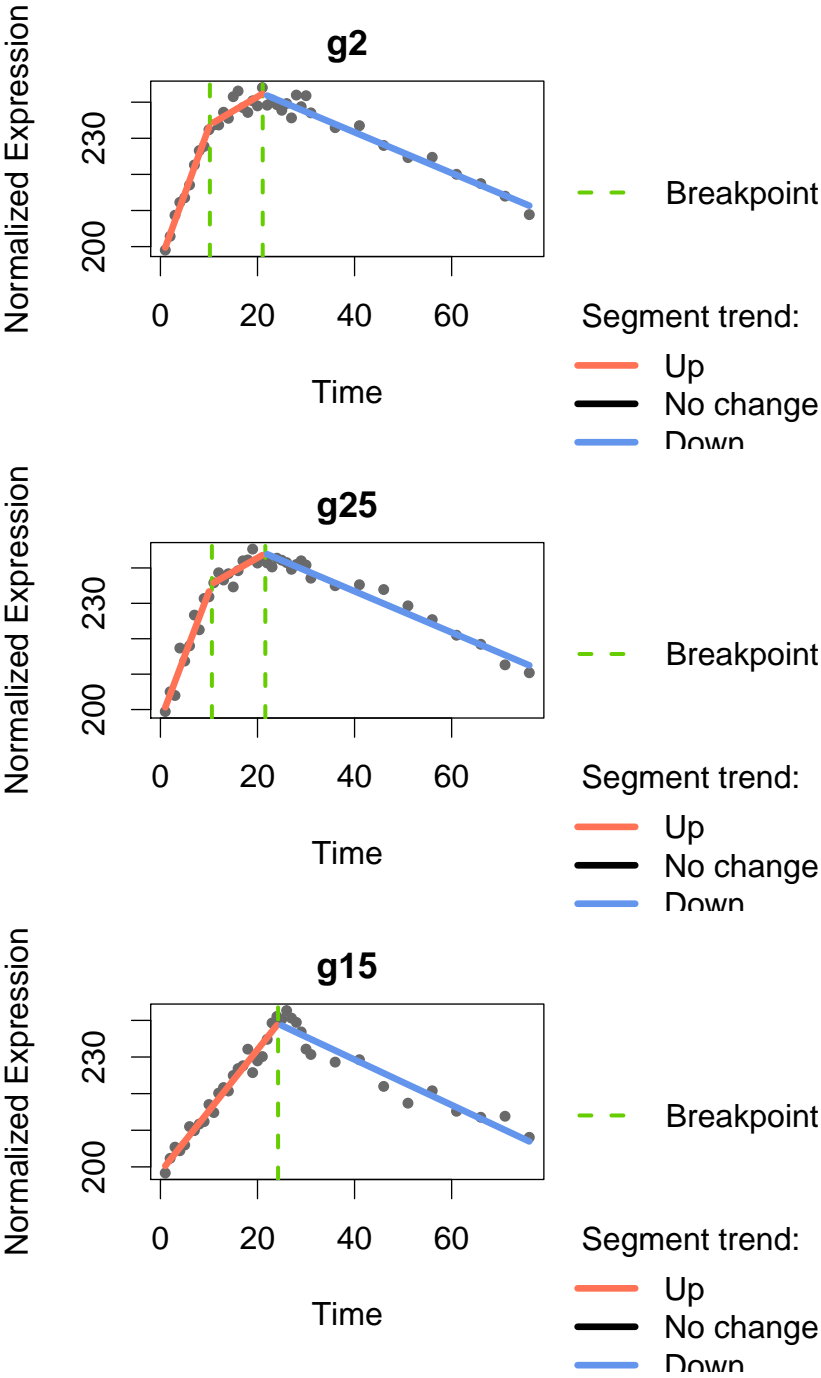
Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



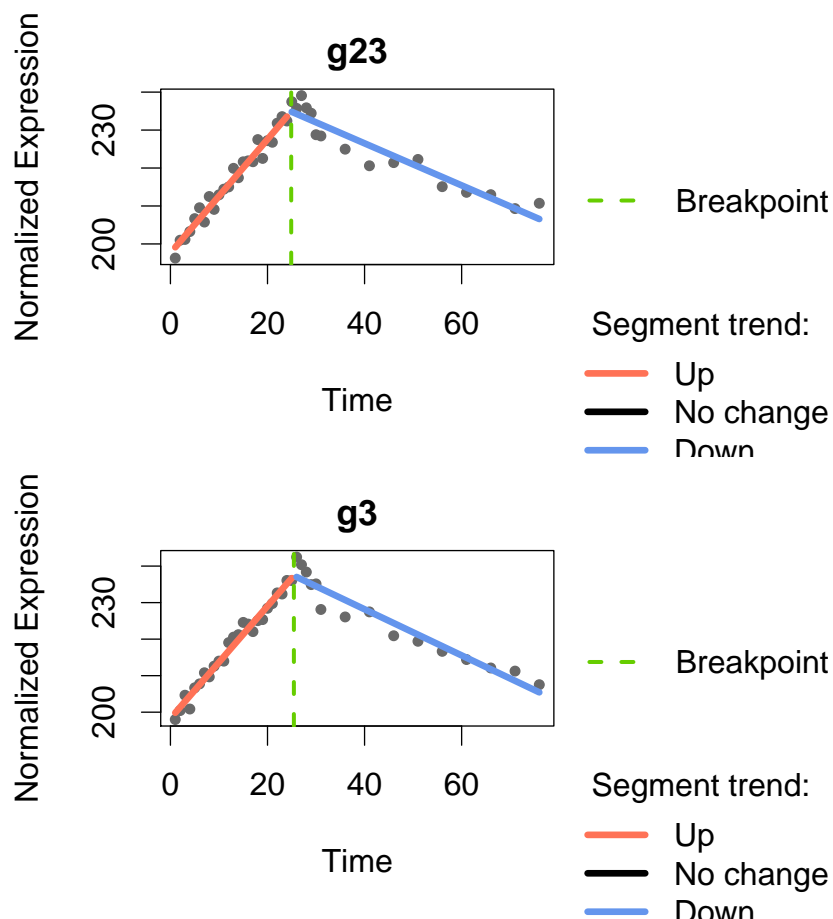
Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments



```
# Genes that are constant, none
pat4 <- extractPattern(res2, Pattern = c("same"))
```

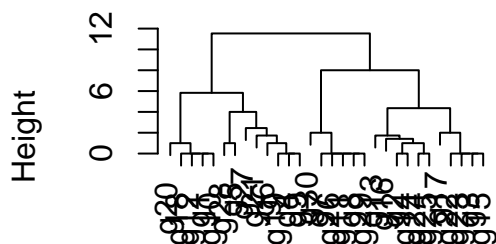
6 Further analysis of Trendy expression trends

For each gene, the Trendy segments are assigned a direction as: "up", "down", or "same". These directions can be used to cluster genes having similar trends along the time-course. Here I will use a simple hierarchical clustering to demonstrate the clustering but other clustering methods may be applicable.

```
# Get trend matrix:
trendMat <- res.top$Trends
# Cluster genes using hierarchical clustering:
hc.results <- hclust(dist(trendMat))
plot(hc.results) #decide how many cluster to choose
```


Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments

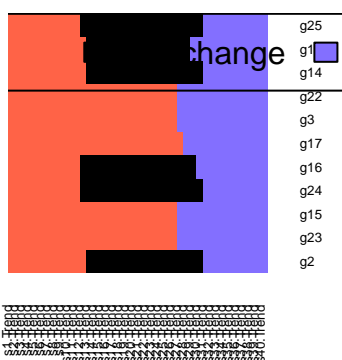
Cluster Dendrogram



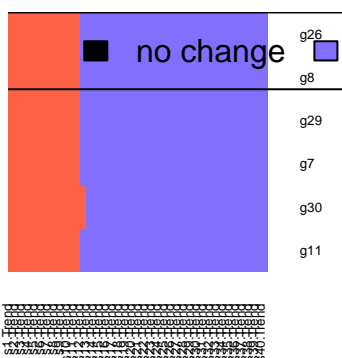
```
dist(trendMat)
hclust (*, "complete")
```

```
#Let's say there are 4 main clusters
hc.groups <- cutree(hc.results, k = 4)

cluster1.genes <- names(which(hc.groups == 1))
res.trend2 <- trendHeatmap(res.top, featureNames = cluster1.genes)
```



```
cluster4.genes <- names(which(hc.groups == 4))
res.trend2 <- trendHeatmap(res.top, featureNames = cluster4.genes)
```



The genes in each cluster can then be input to a gene enrichment analysis, using tools such as enrichr, allez, or GSEA.

Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments

7 Additional options

In the `trendy` function, the thresholds c_{num} and c_{pval} can be specified via parameters `minNumInSeg` and `pvalCut`, respectively.

8 Trendy shiny app

The Trendy shiny app requires an `.RData` object output from the `trendy` function, which can be obtained by setting `saveObject=TRUE`.

```
res <- trendy(trendyExampleData, maxK=2, saveObject = TRUE, fileName="exampleObject")
res <- results(res)
```

Then in R run:

```
trendyShiny()
```

Trendy

Upload `.RData` object first, then obtain list of genes according to some pattern or visualize genes one by one.

Input `.Rdata` from `trendy()` run:

Browse...	exampleObject_trendyForShin
Upload complete	

Upload File

File is uploaded!

Figure 1: Upload shiny object

Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments

Trendy

Upload .RData object first, then obtain list of genes according to some pattern or visualize genes one by one.

Input .Rdata from trendy() run:

Browse...

exampleObject_trendyForSh

Upload complete

Upload File

File is uploaded!

Obtain gene patterns

[Visualize genes](#)

Please select a folder for output :

Select Output Folder

Enter pattern (separate by comma, no spaces):

up,down

Only consider genes with adjusted R squared greater than:

.5

Only consider genes with pattern after time-point:

0

Output a plot of patterned genes?

☒ Yes

☐ No

Output file name (will default to pattern)

Submit for processing

Figure 2: [Find all genes with a given pattern](#)

Trendy: Segmented regression analysis of expression dynamics for high-throughput time-course experiments

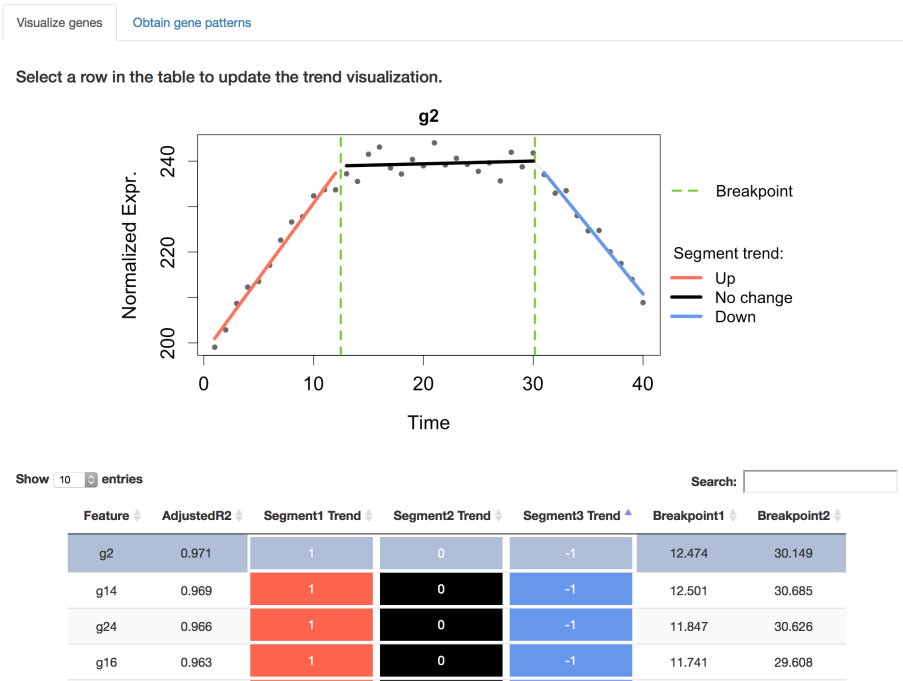


Figure 3: Search genes individually

9 SessionInfo

```
sessionInfo()

## R version 3.5.0 (2018-04-23)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.4 LTS
##
## Matrix products: default
## BLAS: /home/biocbuild/bbs-3.7-bioc/R/lib/libRblas.so
## LAPACK: /home/biocbuild/bbs-3.7-bioc/R/lib/libRlapack.so
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8      LC_COLLATE=C
##  [5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8     LC_NAME=C
##  [9] LC_ADDRESS=C             LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods   base
##
## other attached packages:
## [1] gplots_3.0.1 Trendy_1.2.2
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.17      formatR_1.5
##  [3] compiler_3.5.0    later_0.7.3
##  [5] highr_0.7         GenomeInfoDb_1.16.0
##  [7] XVector_0.20.0    zlibbioc_1.26.0
##  [9] bitops_1.0-6      tools_3.5.0
## [11] digest_0.6.15     lattice_0.20-35
## [13] evaluate_0.10.1   Matrix_1.2-14
## [15] DelayedArray_0.6.1 shiny_1.1.0
## [17] yaml_2.1.19       parallel_3.5.0
## [19] GenomeInfoDbData_1.1.0 stringr_1.3.1
## [21] knitr_1.20        caTools_1.17.1
## [23] gtools_3.5.0      S4Vectors_0.18.3
## [25] shinyFiles_0.6.2  IRanges_2.14.10
## [27] grid_3.5.0        stats4_3.5.0
## [29] segmented_0.5-3.0 rprojroot_1.3-2
## [31] Biobase_2.40.0    R6_2.2.2
## [33] BiocParallel_1.14.1 rmarkdown_1.10
## [35] RJSONIO_1.3-0     gdata_2.18.0
## [37] magrittr_1.5      matrixStats_0.53.1
## [39] backports_1.1.2   promises_1.0.1
## [41] htmltools_0.3.6   BiocGenerics_0.26.0
## [43] GenomicRanges_1.32.3 SummarizedExperiment_1.10.1
## [45] BiocStyle_2.8.2   mime_0.5
## [47] xtable_1.8-2      httpuv_1.4.4.1
## [49] KernSmooth_2.23-15 stringi_1.2.3
## [51] RCurl_1.95-4.10
```