

# Package ‘RNAinteractMAPK’

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**Type** Package

**Title** Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi

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**Description**

This package includes all data used in the paper -Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi- by Horn, Sandmann, Fischer et al., Nat. Methods, 2011. The package vignette shows the R code to reproduce all figures in the paper.

**License** Artistic-2.0

**LazyLoad** yes

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**Suggests** qvalue, lattice

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RNAinteractMAPK-package

*Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi.*

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## Description

The package contains the data and the source code to reproduce the results and figures from the paper

T. Horn, T. Sandmann, B. Fischer, W. Huber, M. Boutros. Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi. Nature Methods, 2011.

## Details

See vignette("RNAinteractMAPK") for details.

## Package content

See vignette("RNAinteractMAPK") for more detail on how to obtain the data used for specific figures. In addition this vignette contains the complete analysis and the generation of all figures.

The main screen can be loaded by `data("Dme12PPMAPK", package="RNAinteractMAPK")`. Access to the pairwise interaction data is done via the `getData` function from the `RNAinteract`-package. See example below.

The following datasets are provided with this package:

<code>Dme12PPMAPK</code>	interaction data of main screen. See example below.
<code>ElpB1phenotype</code>	in vivo experiment on ectopic wing vein formation (used in Figure 5f)
<code>mRNAdoubleKDefficiency</code>	mRNA level after double gene knockdown (used in Figure S1)
<code>mRNAsingleKDefficiency</code>	mRNA level after single gene knockdown (used in Figure S2)
<code>singleKDphenotype</code>	single knockdown phenotypes (used in Figure S3)
<code>dsRNAiDilutionSeries</code>	dsRNA dilution series (used in Figure S5)
<code>Networks</code>	known interactions from DroID (used in Figure S12)
<code>pathwayMembership</code>	pathway membership of genes (used in Figure S13)
<code>PhysicalInteractions</code>	Known physical interactions (used in Figure S13)
<code>cellTiterGlo</code>	cellTiterGlo viability data (used in Figure S15)

Within this package a number of specialized functions is defined written for the analysis of the MAPK interaction screen and additional experiments shown in the paper. These functions are not intended to be general purpose analysis functions, but should document the steps of analysis of the paper. Therefore, these functions are not exported. A list of functions is given below. A general purpose package for the analysis of genetic interaction screens is the package `RNAinteract`.

The following functions are provided within this package.

Functions used for the classification: `MAPK.predict.classification`, `MAPK.cv.classifier`, `MAPK.getCV`, `MAPK.ternary.plot`, `MAPK.getXY`, `MAPK.plot.classification`.

Functions for the analysis of the interaction surfaces: `MAPK.plot.TPS.single`, `MAPK.plot.TPS.all`, `MAPK.estimate.TPS`, `MAPK.cv.TPS`, `MAPK.screen.as.array`.

A function to write the hit list: `MAPK.report.gene.lists.paper`.

A function to plot a heatmap: `MAPK.plot.heatmap.raster`.

A function to plot smooth scatters: `MAPK.smooth.scatter`.

### Author(s)

Bernd Fischer

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### References

T. Horn, T. Sandmann, B. Fischer, W. Huber, M. Boutros. Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi. *Nature Methods*, 2011.

### See Also

[RNAinteractMAPK-package](#)

### Examples

```
data(Dme12PPMAPK)
Dme12PPMAPK

# Obtain the pairwise interaction matrix
PI <- getData(Dme12PPMAPK, type="pi", format="targetMatrix", screen="mean", withoutgroups = c("pos", "neg"))
```

---

cellTiterGlo	<i>Comparison of interaction experiment with an cellTiterGlo viability assay</i>
--------------	--

---

### Description

For ten gene pairs genetic interactions are measured. The experiment contains 24 different conditions. These are repeated in each row of the three 384 multi well plates. The data.frame contains the plate annotation as well as the viability readout for the three plates.

### Usage

```
data(cellTiterGlo)
```

**Format**

A data frame with 384 observations on the following 6 variables.

Well a character vector  
dsRNA\_1 a character vector  
dsRNA\_2 a character vector  
plate1 a numeric vector  
plate2 a numeric vector  
plate3 a numeric vector

**Source**

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S15.

**Examples**

```
data(cellTiterGlo)
head(cellTiterGlo)
```

---

Dme12PPMAPK

*The interaction data of the main screen*

---

**Description**

Dme12PPMAPK is an object of class [RNAinteract](#). It contains the raw data, the computed main effects, pairwise interaction scores, p-values and q-values computed by a t-test. The package vignette contains the complete code and documentation for the statistical analysis.

**Usage**

```
data(Dme12PPMAPK)
```

**Format**

An object of class [RNAinteract](#).

**Source**

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, main interaction screen.

**Examples**

```
data(Dme12PPMAPK)
Dme12PPMAPK

# Obtain the pairwise interaction matrix
PI <- getData(Dme12PPMAPK, type="pi", format="targetMatrix", screen="mean", withoutgroups = c("pos", "neg"))
```

---

`dsRNAiDilutionSeries` *dsRNA dilution series*

---

## Description

A dilution series for 6 x 6 gene. For each gene pair all combinations of 8 different concentrations of dsRNA reagent are screened. Three readout channels (nrCells, area, intensity) are available in the `data.frame` `dsRNAiDilutionSeries`. The plate annotation is given in the `data.frame` `dsRNAiDilutionSeriesAnno` and precomputed degrees of freedom for thin plate splines are available in the matrix `dsRNAiDilutionSeriesDF`.

## Usage

```
data(dsRNAiDilutionSeries)
```

## Format

- `dsRNAiDilutionSeries`: A data frame with 2688 observations on the following 3 variables.
  - `nrCells` a numeric vector representing the number of cells readout.
  - `area` a numeric vector representing the are readout.
  - `intensity` a numeric vector representing the intensity readout.
- `dsRNAiDilutionSeriesAnno`: A data frame with 2688 observations on the following 7 variables.
  - `plate` a numeric vector representing the plate number.
  - `well` a numeric vector representing the well.
  - `row` a numeric vector representing the row on plate.
  - `col` a numeric vector representing the column on plate.
  - `targetID1` a numeric vector representing the target number of the first reagent (see targets).
  - `targetID2` a numeric vector representing the target number of the second reagent (see targets).
  - `targets` a `data.frame` representing the 49 target reagents For each target reagent the gene name and the dsRNAi concentration is given.
- `dsRNAiDilutionSeriesDF`: A 6 x 6 matrix with degrees of freedom for thin-plate spline regression.

## Source

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure 1abc and Figure S5.

## Examples

```
data(dsRNAiDilutionSeries)
head(dsRNAiDilutionSeries)
head(dsRNAiDilutionSeriesAnno)
head(dsRNAiDilutionSeriesDF)
```

---

ElpB1phenotype	<i>Ectopic wing vein formation phenotype caused by the EgrElpB1.</i>
----------------	--

---

### Description

Partial suppression of ectopic wing vein formation (in vivo fly phenotype) caused by the EgrElpB1 in Cka heterozygous mutant backgrounds. The wing vein formation phenotype is classified as strong and notstrong. It is tested for three fly mutants.

### Usage

```
data(ElpB1phenotype)
```

### Format

A data frame with 3 observations on the following 2 variables.

strong a numeric vector

notstrong a numeric vector

### Source

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure 5f.

### Examples

```
data(ElpB1phenotype)
ElpB1phenotype
```

---

MAPK.cv.classifier	<i>A classifier for genetic interaction data.</i>
--------------------	---

---

### Description

These functions implement a classifier to classify three classes of pathway membership of the RasMAPK and JNK pathway. For each sample and each channel a sparse linear discriminat classifier is trained. The posterior probabilities are averaged over all single classifiers. The classification posterior probabilities of three classes are plotted as a ternary plot (ternary plot adapted from CRAN package vcd).

### Usage

```
MAPK.cv.classifier(sgi, traingroups)
MAPK.predict.classification(sgi, traingroups)
MAPK.plot.classification(posterior,
                        classes = NULL, classnames = NULL,
                        col = "darkgray", y = NULL,
                        classcol = NULL,
                        main = "predicted classification probabilities",
                        pop = TRUE, threshText = 0.3,
                        textToLeft = NULL, textToRight = NULL)
```

## Arguments

<code>sgi</code>	An object of class <a href="#">RNAinteract</a>
<code>traingroups</code>	A list of gene names for the training examples. For each class there should be a vector of gene names.
<code>posterior</code>	A matrix of posterior probabilities. Each row represents one gene, each column represents one class.
<code>classes</code>	The three classes to be displayed on the ternary plot.
<code>classnames</code>	The class names to be displayed.
<code>col</code>	The color used for the text labels.
<code>y</code>	A factor representing the class label for each gene in posterior.
<code>classcol</code>	The color used for the three classes.
<code>main</code>	The title of the plot.
<code>pop</code>	If TRUE, all viewports are popped before finishing the function.
<code>threshText</code>	A threshold for the posterior probability of the three classes. Only genes that are assigned with a larger probability to the three classes are shown.
<code>textToLeft</code>	These text labels will be shown on the left hand side.
<code>textToRight</code>	These text labels will be shown on the right hand side.

## Details

The code for the ternary plot (used by `MAPK.plot.classification`) is adapted from the function `ternaryplot` in the CRAN package `vcd`. Author of the original code is David Meyer ([David.Meyer@R-project.org](mailto:David.Meyer@R-project.org)). References: M. Friendly (2000), *Visualizing Categorical Data*. SAS Institute, Cary, NC. This code is specialized for the publication "Mapping Signalling Networks by RNAi ..." in *Nat. Methods*. It is highly recommended to use the original code by David Meyer.

## Value

`MAPK.cv.classifier` returns a list with the cross validated class assignment probability, as well as the results of the single classifiers.

`MAPK.predict.classifier` returns the predicted posterior probabilities of new genes as well as the classification results of the single classifier.

`MAPK.plot.classifier` returns nothing.

## Author(s)

Bernd Fischer

## References

function `ternaryplot`, CRAN package `vcd`. M. Friendly (2000), *Visualizing Categorical Data*. SAS Institute, Cary, NC.

## See Also

[RNAinteract](#), [RNAinteractMAPK](#)

---

MAPK.estimate.TPS      *genetic interaction surfaces*

---

## Description

Genetic interaction surfaces are estimated from a dilution experiment. Cells are treated with two RNAi's. The concentration of the RNAi reagent is changed in 8 steps. All 8 x 8 combinations of concentrations are tested for 6 x 6 gene pairs.

## Usage

```
MAPK.screen.as.array(data, Anno)
MAPK.estimate.TPS  (A, DF, n.out = 8, channel = 1)
MAPK.cv.TPS       (A, range.df = 6:56, channel = 1)
MAPK.plot.TPS.all (TPSmodel, range = c(-6, 6), fill = c("cornflowerblue",
  "cornflowerblue", "black", "#777700", "yellow"),
  channel = 1)
MAPK.plot.TPS.single(gene1, gene2, TPSmodel, range = c(-6, 6),
  fill = c("cornflowerblue", "cornflowerblue", "black",
  "#777700", "yellow"), channel = 1)
```

## Arguments

	<code>data, Anno</code>	A data.frame containing the read.out of the dilution screen. Each row is one well. Each column one feature.
<code>data0</code>		A data.frame containing the plate configuration. For each row in data there should a row in Anno.
<code>A</code>		An array of dimension concentration x concentration x genes x genes x channel as returned by <code>MAPK.screen.as.array</code> .
<code>DF</code>		A 6 x 6 matrix of degrees of freedom for the thin spline plate regression.
<code>n.out</code>		number of points for sampling from the regression function.
<code>channel</code>		The channel used.
<code>range.df</code>		The range of degrees of freedom that is considered for cross validation.
<code>range</code>		The range of pairwise interaction scores that is shown by the colorbar.
<code>gene1, gene2</code>		The genes for which the interaction surface is plotted.
<code>TPSmodel</code>		The TPS model estimated by <code>MAPK.estimate.TPS</code>
<code>fill</code>		The range of colors used for the color code.

## Details

The screen readout can be reshaped as an array with dimensions concentration x concentration x genes x genes x channel by the function `MAPK.screen.as.array`. Then the function `MAPK.estimate.TPS` fits a regression in the 8 x 8 pairwise dilution series. The degrees of freedom for the regression can be estimated automatically by cross validation with the function `MAPK.cv.TPS`. Finally one can plot the interaction surface for a single gene or an overview of interaction surfaces for all genes with the functions `MAPK.plot.TPS.single` or `MAPK.plot.TPS.all`.



**Value**

- MAPK.screen.as.array returns an array of dimensions concentration x concentration x genes x genes x channel with the screen data.
- MAPK.estimate.TPS returns a regression model estimated by thin plate splines for each pair of genes and subsampled matrices.
- MAPK.cv.TPS.all returns
  - DF a matrix with degrees of freedom.
  - CVeror The prediction error estimated by cross validation.
  - CVerorSD The standard deviation of the prediction error estimated by cross validation.
- MAPK.plot.TPS.single,MAPK.plot.TPS.all: An object of class "trellis". See [levelplot](#) for details.

**Author(s)**

Bernd Fischer

**See Also**

[RNAinteract-package](#), [RNAinteractMAPK-package](#)

---

MAPK.plot.heatmap.raster

*Plots a heatmap using grid.raster*

---

**Description**

This functions provides a grid plot that displays the raster image of a heatmap without any axis or label. This function is adapted from the function [grid.sgiHeatmap](#) from the package [RNAinteract-package](#). It is highly recommended to use the original function [grid.sgiHeatmap](#).

**Usage**

```
MAPK.plot.heatmap.raster(X, subset = NULL,
                          hc.row = NULL, hc.col = NULL,
                          pi.max = NULL)
```

**Arguments**

X	A matrix of pairwise interaction scores.
subset	A subset of genes that are displayed in the rows.
hc.row	A hclust object.
hc.col	A hclust object.
pi.max	The maximum interaction score of the colorbar. All interaction scores larger than this value will be displayed in the same color.

**Value**

Nothing is returned.

**Author(s)**

Bernd Fischer

**See Also**[RNAinteract-package](#), [RNAinteractMAPK-package](#)

---

MAPK.report.gene.lists.paper  
*A hitlist report.*

---

**Description**

Reports the hitlist of genetic interactions, with p-values from a t-test with pooled variance estimate, from limma, and from Hotelling T<sup>2</sup> test.

**Usage**

```
MAPK.report.gene.lists.paper(sgi, sgilimma, sgi3T2, screen = "mean")
```

**Arguments**

sgi	An object of class <a href="#">RNAinteract</a> containing p-values from a t-test with pooled variance estimate.
sgilimma	An object of class <a href="#">RNAinteract</a> containing p-values from limma.
sgi3T2	An object of class <a href="#">RNAinteract</a> containing p-values from a Hotelling T <sup>2</sup> test.
screen	The screen name for which the report should be written.

**Details**

Writes tab-separated lists for each single test as well as a joint table with all three tests.

**Value**

Nothing is returned.

**Author(s)**

Bernd Fischer

**See Also**[RNAinteract-package](#), [RNAinteractMAPK-package](#)

---

MAPK.smooth.scatter     *smooth scatter using grid raster*

---

### Description

This function is a reimplementaion of [smoothScatter](#). For nicer graphics output the background image is written by [grid.raster](#). It is recommended to use the [smoothScatter](#) function from the [graphics](#) package.

### Usage

```
MAPK.smooth.scatter(x, y, n = 75,
                    nrpoints = 100, col = "blue",
                    pch = 20, size = unit(0.3, "char"), cex = 1.2,
                    colramp = colorRampPalette(c("white", "blue", "green", "yellow", "red"))(256),
                    xlab = "", ylab = "", respect = FALSE)
```

### Arguments

x	x-values.
y	y-values. Has to be the same length as x.
n	nr of bins used for the kernel density estimation.
nrpoints	nrpoints points in the lowest density region will be plotted. This allows the identification of outliers.
col	color of points.
pch	symbol to plot points.
size	The size of the points.
cex	The size of the label text.
colramp	color ramp for the density plot.
xlab,ylab	axis labels.
respect	A logical value indicating if the height and width of the axis scales should respect each other.

### Details

Plots a density plot with [grid](#) graphics.

### Author(s)

Bernd Fischer

### See Also

[RNAinteractMAPK-package](#), [smoothScatter](#)

---

 mRNAdoubleKDefficiency

*mRNA levels for double knock downs*


---

### Description

qPCR measurements for the mRNA level after a double gene knock down (ratio relative to wild type control). The experiments tests the knock down efficiency in the presence of a second gene knock down.

### Usage

```
data(mRNAdoubleKDefficiency)
```

### Format

A data frame with 320 observations on the following 5 variables.

`template` an ordered factor with levels Fluc < CG10417 < CG13197 < CG9391 < egr < lic < PRL-1 < Rho1 < Tak1

`query` a factor with levels CG10417 [query dsRNA] CG13197 [query dsRNA] CG9391 [query dsRNA] egr [query dsRNA] lic [query dsRNA] PRL-1 [query dsRNA] Rho1 [query dsRNA] Tak1 [query dsRNA]

`qPCR.target` a factor with levels CG10417 CG13197 CG9391 egr lic PRL-1 Rho1 Tak1

`passage` a factor with levels passage 4 passage 42

`RNAi` a numeric vector

### Source

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S2.

### Examples

```
data(mRNAdoubleKDefficiency)
head(mRNAdoubleKDefficiency)
```

---

 mRNAsingleKDefficiency

*mRNA levels for single gene knock downs*


---

### Description

qPCR measurements for the mRNA level after a single gene knock down (ratio relative to wild type control). The experiments is done for two independent designs of RNAi reagents.

### Usage

```
data(mRNAsingleKDefficiency)
```

**Format**

A data frame with 89 observations on the following 5 variables.

Symbol a character vector  
MeanDesign1 a numeric vector  
StderrDesign1 a numeric vector  
MeanDesign2 a numeric vector  
StderrDesign2 a numeric vector

**Source**

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S1.

**Examples**

```
data(mRNAsingleKDefficiency)
head(mRNAsingleKDefficiency)
```

---

Networks

*Knock interaction networks*

---

**Description**

This dataset is a subset of the DroID database. It contains the known (genetic) interactions between the genes regarded in the main screen.

**Usage**

```
data(Networks)
```

**Format**

A data frame with 402 observations on the following 5 variables.

gene1 a character vector  
gene2 a character vector  
correlation a numeric vector  
genetic a numeric vector  
human a numeric vector

**Source**

Data as used in Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S12.

The data is a subset from the drosophila interactions database (DroID), <http://www.droidb.org>, Data version 2010\_10 updated 20 October 2010.

Murali T, Pacifico S, Yu J, Guest S, Roberts GG 3rd, Finley RL Jr . DroID 2011: a comprehensive, integrated resource for protein, transcription factor, RNA and gene interactions for Drosophila. Nucleic Acids Res. 2010 Oct 29.

**Examples**

```
data(Networks)
head(Networks)
```

---

```
pathwayMembership
```

*Pathway membership of genes.*

---

**Description**

The membership of the tested genes in the four pathways JAK/STAT, RasMAPK, JNK, and p38.

**Usage**

```
data(pathwayMembership)
```

**Format**

The format is: chr "pathwayMembership"

**Source**

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S13.

**Examples**

```
data(pathwayMembership)
head(pathwayMembership)
```

---

```
PhysicalInteractions
```

*Known physical interactions*

---

**Description**

This dataset contains a collection of known physical interactions assembled from the literature. It contains the known pathway structure of the RasMAPK and the JNK pathway.

**Usage**

```
data(PhysicalInteractions)
```

**Format**

A data frame with 29 observations on the following 2 variables.

V1 a character vector

V2 a character vector

**Source**

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S13.

**Examples**

```
data(PhysicalInteractions)
head(PhysicalInteractions)
```

---

singleKDphenotype	<i>Single knockdown phenotype</i>
-------------------	-----------------------------------

---

**Description**

This data.frame singleKDphenotype contains a screen assessing the single knock down phenotypes (nrCells, intensity, and area) of the tested genes. singleKDphenotypeAnno is a data.frame describing the plate annotation.

**Usage**

```
data(singleKDphenotype)
```

**Format**

The format is: chr "singleKDphenotype" chr "singleKDphenotypeAnno"

**Source**

Horn, Sandmann, Fischer, Huber, Boutros, Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi, Nature Methods, 2011, Figure S3.

**Examples**

```
data(singleKDphenotype)
head(singleKDphenotype)
head(singleKDphenotypeAnno)
```

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