

# Package ‘DArand’

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**Title** Differential Analysis with Random Reference Genes

**Version** 0.0.1.2

**Description** Differential Analysis of short RNA transcripts that can be modeled by either Poisson or Negative binomial distribution. The statistical methodology implemented in this package is based on the random selection of references genes (Desaulle et al. (2021) <[doi:10.48550/arXiv.2103.09872](https://doi.org/10.48550/arXiv.2103.09872)>).

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build\_example

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*Simulation of gene expressions using independant negative binomials*


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## Description

Simulation of gene expressions using independant negative binomials

## Usage

```
build_example(
  m = 500,
  m1,
  n1 = 6,
  n2 = n1,
  fold = 100,
  mu0 = 100,
  use.scales = FALSE,
  nb.size = Inf
)
```

## Arguments

m	number of genes
m1	number of differentially expressed genes. In the expression matrix, m1 first columns contain differentially expressed genes.
n1	number of samples under the first condition. The first n1 rows in the expression matrix.
n2	number of samples under the second condition (default n2=n1)
fold	maximal fold change added to the first m1 genes. The fold decreases proportionally to $1/\sqrt{1:m1}$ .
mu0	mean relative expression
use.scales	if TRUE random scales are used, otherwise all scales are set to 1.
nb.size	number of successful trials in the negative binomial distribution. If nb.size is set to Inf (default), the Poisson model is used.

## Details

The function generates a list, of which the first element X is a matrix of n1+n2 and m dimension with simulated expressions under Poisson or Negative Binomial distribution. Lines 1:n1 correspond to the first condition (or sub-group) and lines (n1+1):(n1+n2) to the second one. Columns 1:m1 contain counts imitating differential expressions.

In the ideal situation there is no microscopical variability between samples and all scales (so-called scaling factors) would be the same. To simulate examples corresponding to this perfect situation, use argument use.scales=FALSE which will set all scales to 1. When use.scales=TRUE, scales are simulated under uniform distribution  $Unif(0.25,4)$ .

The fold is maximal for the first expression and decreases proportionally to  $1/\sqrt{1:m1}$ . The smallest fold  $\text{fold}/\sqrt{m1}$  is set to the  $m1$ -th expression.

### Value

A list with components

`X` a two-dimensional array containing the expression table of `n` individuals in rows and `m` gene expressions in columns.

`m1` number of differentially expressed genes (as in arguments).

`n1` number of samples under the first condition (as in arguments).

`n2` number of samples under the second condition (as in arguments).

`fold` maximal fold change between the differentially expressed genes and invariant genes (as in arguments).

`scales` vector of simulated scales.

`mu0` mean relative expression (as in arguments).

### Examples

```
L = build_example(m=500,m1=25,n1=6,fold=20,mu0=100,use.scales=FALSE,nb.size=Inf)
```

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DArand

*Do Differential Analysis with Random Reference Genes*


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### Description

Implement the DArand procedure for transcriptomic data. The procedure is based on random and repeated selection of subsets of reference genes as described in the paper cited below. Observed counts data are normalized with counts from the subset and a differential analysis is used to detect differentially expressed genes. Through repetitions, the number times a gene is detected is recorded and the final selection is determined from p-values computed under Binomial distribution and adjusted with the Holm's correction.

### Usage

```
DArand(
  X,
  n1,
  k = NULL,
  alpha = 0.05,
  eta = 0.05,
  beta = 0.1,
  r = 1000,
  with.info = FALSE,
  clog = 1,
  use.multi.core = TRUE,
```

```

    step = 0,
    scales = NULL,
    use.Iter = TRUE,
    set.seed = NULL
  )

```

### Arguments

<code>X</code>	a two-dimensional array (or <code>data.frame</code> ) containing the expression table of <code>n</code> individuals in rows and <code>m</code> gene expressions in columns.
<code>n1</code>	integer, number of individuals of the first category, should be smaller than <code>n</code>
<code>k</code>	integer, number of random genes selected (default <code>k = ceiling(log2(m))</code> ) as reference genes.
<code>alpha</code>	numeric, global test level (default 0.05)
<code>eta</code>	numeric, inner test level (default 0.05)
<code>beta</code>	numeric, inner type II error (default 0.1)
<code>r</code>	integer, number of random 'reference' set selected (the default 1000)
<code>with.info</code>	logical, if TRUE results are displayed (the default FALSE)
<code>clog</code>	numeric, constant (default 1) controlling the gaussian approximation of the test statistic (in Negative Binomial and Poisson case) .
<code>use.multi.core</code>	logical, if TRUE (the default) parallel computing with <code>mclapply</code> is used.
<code>step</code>	integer, only used when <code>use.Iter</code> is TRUE to get information on the number of iterations (default 0). Not for use.
<code>scales</code>	numeric, only used for simulation of oracle purpose (default NULL). Not for use.
<code>use.Iter</code>	logical, applies iterative procedure (default FALSE)
<code>set.seed</code>	numeric, set random seed (as is in <a href="#">set.seed</a> function for random number generation ), here default is NULL.

### Details

The expression table should be organized in such a way that individuals are represented in rows and genes in columns of the `X` array. Furthermore, in the current version, the procedure provides a differential analysis comparing exactly two experimental conditions. Hence, lines from 1 to `n1` should correspond to the first condition and the remaining lines to the second condition.

In the inner part of the procedure, called further *randomization*, scaling factors are estimated using a normalization subset of `k` genes randomly selected from all `m` genes. These `k` genes are used as reference genes. The normalized data are compared between the experimental conditions within an approximately gaussian test for Poisson or negative-binomial counts as proposed in the methodology cited below. For this inner test the type I (`eta`) and the type II (`beta`) errors should be specified, otherwise the default values will be used. Since true reference genes (*housekeeping genes*) are unknown, the inner part is repeated `r` times.

Through all `r` randomization, for each gene, the number of detections (*i.e.* the number of randomizations when a given gene is identified as differentially expressed) is collected. For these detection counts, the corresponding p-values are computed under the Binomial distribution. The finale detection uses the p-values and, owing to Holm's correction, controls FWER at specified level `alpha`.

The maximal number of discoveries is limited to Delta - the parameter that is a function of eta, beta and the probability of selecting a subset containing at least one differentially expressed gene leading to a wrong normalization (see [select\\_prob](#) ). If use.Iter is TRUE (the default), the maximal number of discoveries is limited (per iteration) to Delta. The procedure is iterated as long as the number of discoveries is equal to the value of Delta computed in the iteration. Starting from step=1, at each iteration the one-type error is halved  $\alpha = \alpha/2$  to ensure the overall test level respects the initial alpha.

clog is a constant that controls gaussian approximation of the test statistic for the count data arising from Negative Binomial or Poisson distribution. The constant should be adjusted to keep the probability  $1 - 5 \cdot n^{(-clog)}$  high while shift term  $1 + \sqrt{clog \cdot n}$  low.

### Value

position vector of the gene expressions found as differentially expressed.

### Author(s)

D. Desaulle and Y. Rozenholc

### References

*Differential analysis in Transcriptomic: The strength of randomly picking 'reference' genes*, D. Desaulle, C. Hoffman, B. Hainque and Y. Rozenholc. <https://arxiv.org/abs/2103.09872>

### Examples

```
L = build_example(m=500,m1=25,n1=6,fold=20,mu0=100,use.scales=FALSE,nb.size=Inf)
DArand(L$X,L$n1,alpha=0.05)
```

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select_prob	<i>Probabilities to select a normalization set without DE-gene</i>
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### Description

Probabilities to select a normalization set without DE-gene

### Usage

```
select_prob(m, k, invariant = TRUE)
```

### Arguments

m	number of genes
k	normalization subset size
invariant	boolean, when TRUE, probability of selection is evaluated for invariant gene

**Value**

a vector of probabilities of having at least one differential expression used as an reference selected in the normalization subset for any number of differential expressions *d* in the gene collection.

**Examples**

```
select_prob(500, 10, invariant=TRUE)
```

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