

LMGene User's Guide

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1 Introduction

This article introduces usage of the **LMGene** package. **LMGene** has been developed mainly for analysis of microarray data using a linear model and glog data transformation in the R statistical package.

2 Data preparation

LMGene takes objects of class **ExpressionSet**, which is the standard data structure of the **Biobase** package. Hence, if data which is of class **ExpressionSet** already, the user can jump to further steps, like diagnostic plotting or g-log transformation. Otherwise, the user needs to generate new objects of class **ExpressionSet**. For more detail, please see the vignette, ‘Textual Description of Biobase’ in the **Biobase** package.

Note: ExpressionSet. In this package, an object of class **ExpressionSet** must produce proper data using the commands `exprs(object)` and `phenoData(object)`.

Example. **LMGene** includes a sample array data which is of class **ExpressionSet**. Let's take a look this sample data.

1. First, load the necessary packages in your R session.

```
> library(LMGene)
> library(Biobase)
> library(tools)
```

2. Load the sample **ExpressionSet** class data in the package **LMGene**.

```
> data(sample.eS)
```

3. View the data structure of the sample data and the details of `exprs` and `phenoData` slots in the data.

```
> slotNames(sample.eS)
```

```
[1] "assayData"      "phenoData"      "featureData"
[4] "experimentData" "annotation"      "protocolData"
[7] ".__classVersion__"
```

```
> dim(exprs(sample.eS))
```

```
[1] 613 32
```

```
> exprs(sample.eS)[1:3, ]
```

	p1d0	p1d1	p1d2	p1d3	p2d0	p2d1	p2d2	p2d3	p3d0	p3d1	p3d2	p3d3	p4d0	p4d1	p4d2
g1	216	149	169	113	193	172	167	168	151	179	142	156	160	214	157
g2	334	311	187	135	514	471	219	394	367	390	365	387	318	378	329
g3	398	367	351	239	712	523	356	629	474	438	532	427	429	574	419

	p4d3	p5d0	p5d1	p5d2	p5d3	p6d0	p6d1	p6d2	p6d3	p7d0	p7d1	p7d2	p7d3	p8d0	p8d1
g1	195	165	144	185	162	246	227	173	151	796	378	177	278	183	285
g2	450	293	285	390	428	645	631	324	343	852	451	259	379	259	386
g3	564	438	321	519	488	824	579	416	489	1046	501	375	388	373	509

	p8d2	p8d3
g1	275	202
g2	361	333
g3	468	436

```
> phenoData(sample.eS)
```

```
An object of class "AnnotatedDataFrame"
sampleNames: p1d0, p1d1, ..., p8d3 (32 total)
varLabels and varMetadata description:
  patient: patient
  dose: dose
```

```
> slotNames(phenoData(sample.eS))
```

```
[1] "varMetadata"      "data"              "dimLabels"
[4] ".__classVersion__"
```

Data generation. If you don't have `ExpressionSet` class data, you need to make some. `LMGene` provides a function that can generate an object of class `ExpressionSet`, assuming that there are array data of `matrix` class and experimental data of `list` class.

1. The package has sample array and experimental data, `sample.mat` and `vlist`.

```

> data(sample.mat)
> dim(sample.mat)

[1] 613  32

> data(vlist)
> vlist

$patient
[1] 1 1 1 1 2 2 2 2 3 3 3 3 4 4 4 4 5 5 5 5 6 6 6 6 7 7 7 7 8 8 8 8
Levels: 1 2 3 4 5 6 7 8

$dose
[1] 0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3

```

2. Generate ExpressionSet class data using `neweS` function.

```

> test.eS <- neweS(sample.mat, vlist)
> class(test.eS)

[1] "ExpressionSet"
attr(,"package")
[1] "Biobase"

> identical(sample.eS, test.eS)

[1] FALSE

```

c.f. If you have different types of array data, such as `RGList`, `marrayRaw`, and so on, you can convert them into `ExpressionSet` class by using `as` method after installing `convert` package.

3 G-log transformation

1. Estimating parameters for g-log transformation. The linear model is not applied to the raw data, but to transformed and normalized data. Many people use a log transform. LMGene uses a log-like transform involving two parameters. We estimate the parameters λ and α of the generalized log transform $\log(y - \alpha + \sqrt{(y - \alpha)^2 + \lambda}) = \sinh^{-1}(\frac{y - \alpha}{\lambda}) + \log(\lambda)$ using the function `tranest` as follows:

```

> tranpar <- tranest(sample.eS)
> tranpar

$lambda
[1] 726.6187

$alpha
[1] 56.02754

```

The optional parameter `ngenes` controls how many genes are used in the estimation. The default is all of them (up to 100,000), but this option allows the use of less. A typical call using this parameter would be

```
> tranpar <- tranest(sample.eS, 100)
> tranpar
```

```
$lambda
[1] 433.8768
```

```
$alpha
[1] 47.60042
```

In this case, 100 genes are chosen at random and used to estimate the transformation parameter. The routine returns a list containing values for `lambda` and `alpha`.

2. **G-log transformation.** Using the obtained two parameters, the g-log transformed expression set can be calculated as follows.

```
> trsample.eS <- transeS(sample.eS, tranpar$lambda, tranpar$alpha)
> exprs(sample.eS)[1:3, 1:8]
```

	p1d0	p1d1	p1d2	p1d3	p2d0	p2d1	p2d2	p2d3
g1	216	149	169	113	193	172	167	168
g2	334	311	187	135	514	471	219	394
g3	398	367	351	239	712	523	356	629

```
> exprs(trsample.eS)[1:3, 1:8]
```

	p1d0	p1d1	p1d2	p1d3	p2d0	p2d1	p2d2	p2d3
g1	5.823290	5.322603	5.499515	4.898110	5.677725	5.523583	5.483146	5.491363
g2	6.351855	6.268379	5.636027	5.177544	6.838688	6.742068	5.840817	6.541643
g3	6.553104	6.460652	6.409374	5.950458	7.192277	6.857782	6.425682	7.058906

3. **Tranest options:** `multiple alpha`, `lowessnorm`, `model`

Rather than using a single `alpha` for all samples, we can estimate a separate `alpha` for each sample. This allows for differences in chips, in sample concentration, or exposure conditions.

```
> tranparamult <- tranest(sample.eS, mult = TRUE)
> tranparamult
```

```
$lambda
[1] 689.2819
```

```
$alpha
[1] 69.67146 37.02711 54.13904 69.35728 60.33270 60.75301 71.72965
[8] 64.55506 58.63427 65.73625 48.40173 59.43778 76.34568 78.81046
```

```
[15] 82.20326 96.19938 77.60070 79.48089 73.63257 73.41650 33.86029
[22] 69.26448 55.75460 54.29840 139.89493 91.36521 46.46158 59.02056
[29] 73.60255 89.48728 57.13887 64.98866
```

For vector alphas, transeS uses exactly the same syntax:

```
> trsample.eS <- transeS(sample.eS, tranparmult$lambda, tranparmult$alpha)
> exprs(trsample.eS)[1:3, 1:8]
```

```
      p1d0    p1d1    p1d2    p1d3    p2d0    p2d1    p2d2    p2d3
g1 5.686954 5.424873 5.449682 4.549380 5.590642 5.418542 5.268332 5.347915
g2 6.272797 6.308464 5.592073 4.915159 6.811348 6.710929 5.693269 6.492140
g3 6.488757 6.493737 6.388361 5.832776 7.173087 6.830052 6.345199 7.029530
```

It's also possible to estimate the parameters using the more accurate lowess normalization (as opposed to uniform normalization):

```
> tranparmult <- tranest(sample.eS, ngenes = 100, mult = TRUE,
+   lowessnorm = TRUE)
> tranparmult
```

```
$lambda
[1] 580.0284
```

```
$alpha
[1] 80.68571 54.56052 60.87438 70.70900 64.51954 63.78964 74.61903
[8] 69.01873 62.51259 69.41077 67.46891 61.41619 67.63799 70.67033
[15] 62.56282 87.91923 61.45574 58.38632 64.74360 57.06333 48.35156
[22] 102.50360 54.98847 62.37547 177.51333 101.33924 58.44630 69.00983
[29] 59.56548 83.73135 59.28274 62.43646
```

It is even possible now to estimate parameters using a specified model. For example, if we think that the interaction of variables in vlist is important, we can add interaction to the model:

```
> tranpar <- tranest(sample.eS, model = "patient + dose + patient:dose")
> tranpar
```

```
$lambda
[1] 860.0836
```

```
$alpha
[1] 55.68625
```

The model is always specified in the same way as the right-hand side of an lm model. In the example above, we set the parameters to minimize the mean squared error for a regression of transformed gene expression against patient, log dose, and their interaction.

Be very careful of using interactions between factor variables. If you do not have enough replications, you can easily overfit the data and have no errors to work with.

Naturally, it's possible to use mult, lowessnorm, and model all together.

4 Finding differentially expressed genes

1. **Transformation and Normalization.** Before finding differentially expressed genes, the array data needs to be transformed and normalized.

```
> trsample.eS <- transeS(sample.eS, tranparamult$lambda, tranparamult$alpha)
> ntrsample.eS <- lnormeS(trsample.eS)
```

2. **Finding differentially expressed genes** The lmgene routine computes significant probes using the method of Rocke (2003). A typical call would be

```
> sigprobes <- LMGene(ntrsample.eS)
```

There is an optional argument, level, which is the test level, .05 by default. A call using this optional parameter would look like

```
> sigprobes <- LMGene(ntrsample.eS, level = 0.01)
```

The result is a list whose components have the names of the effects in the model. The values are the significant genes for the test of that effect or else the message "No significant genes".

As with tranest, it's possible to specify a more complex model to LMGene:

```
> sigprobes <- LMGene(ntrsample.eS, model = "patient+dose+patient:dose")
> sigprobes
```

```
$patient
 [1] "g2"  "g3"  "g9"  "g10" "g14" "g15" "g49" "g54" "g84" "g85"
[11] "g86" "g93" "g102" "g123" "g139" "g155" "g178" "g179" "g250" "g256"
[21] "g271" "g277" "g310" "g319" "g327" "g336" "g372" "g375" "g384" "g399"
[31] "g405" "g406" "g407" "g408" "g409" "g410" "g411" "g412" "g413" "g414"
[41] "g415" "g423" "g425" "g460" "g461" "g462" "g463" "g477" "g503" "g520"
[51] "g524" "g528" "g566" "g607" "g612"
```

```
$dose
[1] "No significant genes"
```

```
$`patient:dose`
[1] "No significant genes"
```

The routine LMGene requires the multtest package.

References

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- [2] Durbin, B. and Rocke, D. M. (2003a) “Estimation of transformation parameters for microarray data,” *Bioinformatics*, **19**, 1360–1367.
- [3] Durbin, B. and Rocke, D. M. (2003b) “Exact and approximate variance-stabilizing transformations for two-color microarrays,” submitted for publication.
- [4] Geller, S.C., Gregg, J.P., Hagerman, P.J., and Rocke, D.M. (2003) “Transformation and normalization of oligonucleotide microarray data,” *Bioinformatics*, **19**, 1817–1823.
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- [7] Rocke, D. and Durbin, B. (2003) “Approximate variance-stabilizing transformations for gene-expression microarray data,” *Bioinformatics*, **19**, 966–972.