HowTo BGX

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1 Introduction

This vignette describes how to use *bgx*, a C++ implementation of a Bayesian hierarchical integrated approach to the modelling and analysis of Affymetrix GeneChip arrays. The model and methodology is described in Hein et al, 2005.

There are two ways to run *bgx*: (1) through R and (2) as a standalone binary. Both ways make use of probe level GeneChip data, which you must obtain as GeneChip CEL files.

2 Reading in the CEL files

When you load *bgx*, several required packages from the Bioconductor[1] project are automatically loaded.

```
> library(bgx)
```

The *affy* package allows you to read CEL files into an `AffyBatch` object. This can be achieved by changing your working directory to wherever the CEL files are stored and executing:

```
> aData <- ReadAffy()
```

This will read in the CEL files in alphabetical order and save the data in the `aData` object. Alternatively, you can specify the specific files you would like to read in by adding their paths to the argument list, for example:

```
> aData <- ReadAffy("CEL/choe/chipC-rep1.CEL", "CEL/choe/chipS-rep2.CEL")
```

[1] `http://bioconductor.org`

3 Running BGX through R

A basic execution of the program can be performed by simply passing an `AffyBatch` object as a single parameter to the `bgx` function and saving the result in an `ExpressionSet` object. The result will hold array-specific gene expression values and their corresponding standard errors in `assayData(eset)$exprs` and `assayData(eset)$se.exprs` respectively.

```
> eset <- bgx(aData)
```

A more elaborate scenario would involve splitting the arrays into a number of conditions using the *samplesets* argument[2]; specifying which genes to analyse with the *genes* argument; specifying whether to take into account probe affinity with *probeAff*; setting the number of burn-in and post burn-in runs with the *burnin* and *iter* arguments respectively; setting the set of parameters to save with the *output* argument[3]; and specifying where to save the runs with *rundir*. Execute `help(bgx)` in R for a full explanation of all the parameters.

As an example, let us analyse the `Dilution` data set and save the results in the current working directory (`"."`):

```
> library(affydata)
> library(hgu95av2cdf)
> data(Dilution)
> eset <- bgx(Dilution, samplesets=c(2,2), probeAff=FALSE, burnin=2048, iter=8192,ge
```

The `eset` object will contain gene expression information for each gene under each condition (not necessarily each array). You may obtain the gene expression measure using the `exprs` function. For instance:

```
> exprs(eset)[10:40,] # Shorthand for assayData(eset)\$exprs[10:40,]
```

```
         condition 1 condition 2
947_at       6.55658     6.24880
948_s_at     4.82887     4.41459
949_s_at     4.83150     4.52507
950_at       4.50565     4.22432
951_at       2.28303     3.12455
952_at       1.76115     1.69608
953_g_at     5.28602     4.84581
```

[2]Note that if your `AffyBatch` object contains information on the experimental design in the `phenoData` slot, you do not need to use the *samplesets* argument.

[3]*output* can be set to either "minimal", "trace" or "all". See the documentation for an explanation of what these levels mean

```
954_s_at     6.39044     6.09729
955_at       6.61334     6.34019
956_at       7.00840     6.71031
957_at       4.61623     4.29676
958_s_at     5.54147     5.17050
959_at       0.96296     1.10783
960_g_at     5.18094     4.96274
961_at       1.80088     1.16916
962_at       1.49278     1.44293
963_at       4.55301     4.20171
964_at       4.24098     4.10523
965_at       2.47125     1.42716
966_at       4.51176     4.02216
967_g_at     4.83973     4.61605
968_i_at     3.23997     3.55578
969_s_at     4.73917     4.47357
970_r_at     6.31080     6.17463
971_s_at     2.92644     2.82488
973_at       4.33811     4.09892
974_at       1.80054     1.45679
975_at       4.33284     3.93199
976_s_at     3.15728     3.31421
977_s_at     4.88528     4.55692
978_at       3.19099     2.48567
```

Run `help(ExpressionSet)` in R for more information.

Note that *samplesets* should be set to an array specifying the number of replicates in each condition. If set to `(3,2)`, `bgx` will treat the first three arrays read into R as replicates under condition 1 and the next two as replicates under condition 2. You should make sure that all condition 1 files are read in first and all condition 2 files are read in second by `ReadAffy()`. You may check the order of the samples in your `AffyBatch` object by using the `sampleNames` function:

```
> sampleNames(Dilution)

[1] "20A" "20B" "10A" "10B"
```

4 Running BGX as a standalone binary

Occasionally it may be useful to run `bgx` as a standalone binary from the command line[4]. In this case, you should use the `standalone.bgx` function instead of the `bgx` function.

[4]You can compile it by tweaking 'src/Makefile.standalone' to your specifications and running 'make -f Makefile.standalone' from the 'src' directory.

It takes the same arguments as `bgx`, with the addition of *dirname*, which should specify where you would like to save the input files required by the standalone binary.

```
aData <- ReadAffy() # Read in 6 arrays across two conditions
                    # in alphabetical order
standalone.bgx(aData, samplesets=c(3,3), genes=c(1:650,1000:1200),
  burnin=16384, iter=65536, output="minimal",
  dirname="input-choe3replicates")
```

Once you have saved the input files, you should locate the binary, make sure it is executable[5], and pass the path to the newly created `infile.txt` file as a single argument. For example:

```
./bgx ../input-choe3replicates/infile.txt
```

5 Detailed analysis of the output

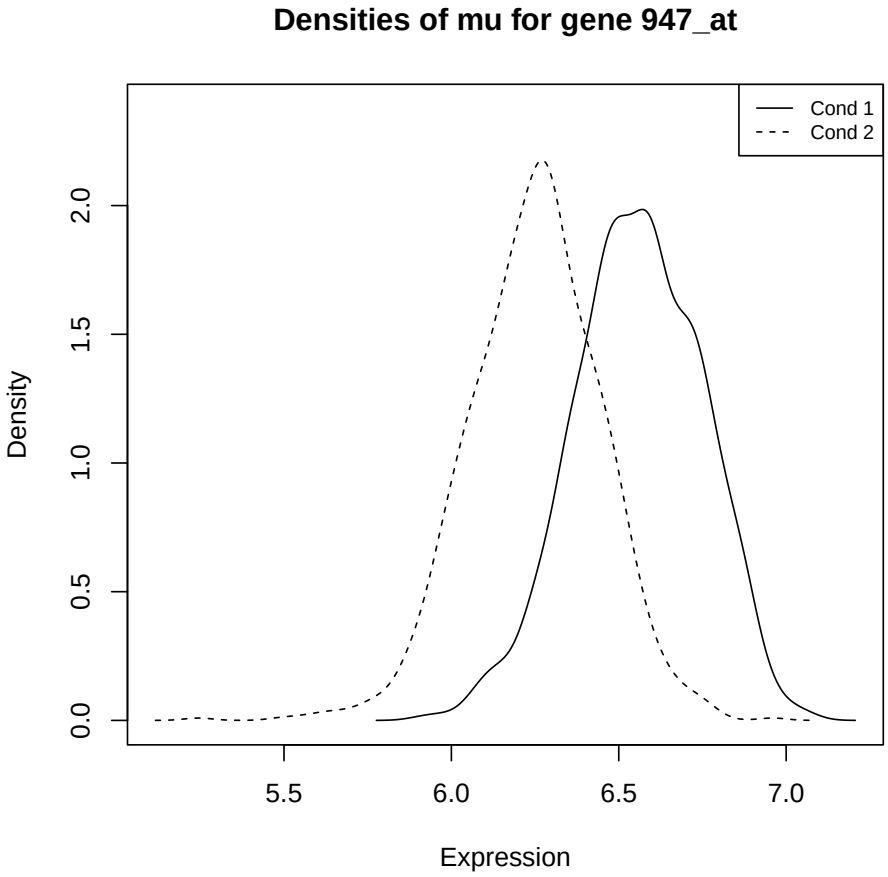
If you wish to analyse the output in detail, you should first read the output into a list as follows:

```
> bgxOutput <- readOutput.bgx("run.1")
```

You may then pass the `bgxOutput` object to any of several analysis functions. For instance, to view the gene expression distributions under the various conditions for gene 10, you could do:

```
> plotExpressionDensity(bgxOutput, gene=10)
```

[5]Under Unix-like environments, you can type `chmod +x bgx` at the command prompt to do this.



In order to get a list of ranked differential expression values, you could do:

```
> rankedGeneList <- rankByDE(bgxOutput)
> print(rankedGeneList[1:25,]) # print top 25 DEG
```

	Position	DiffExpression
955_at	18	37.420388
941_at	4	36.412272
956_at	19	31.903408
AFFX-HSAC07/X00351_5_at	83	31.675462
AFFX-HUMGAPDH/M33197_5_at	89	28.416939
AFFX-HUMGAPDH/M33197_M_at	91	26.989338
947_at	10	25.401802
AFFX-HSAC07/X00351_M_at	85	24.295601
954_s_at	17	21.288885
946_at	9	20.920797
958_s_at	21	18.534143
953_g_at	16	15.174308

```
AFFX-HUMISGF3A/M97935_MA_at       95      15.147713
AFFX-HUMISGF3A/M97935_MB_at       96      14.839909
982_at                            44      14.805079
AFFX-HUMGAPDH/M33197_3_at         87      14.393452
AFFX-BioDn-3_at                   70      13.497089
948_s_at                          11      13.088445
949_s_at                          12      11.003962
957_at                            20      10.978025
AFFX-HUMISGF3A/M97935_3_at        93      10.345521
977_s_at                          39      10.079847
963_at                            26       9.595199
966_at                            29       8.188710
969_s_at                          32       8.120998
```

Run `help(analysis.bgx)` for more detailed usage instructions on the analysis functions.