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, genes = c(12500:12599), output = "all")
```

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, ]
```

```
         condition 1 condition 2
947_at       6.54870     6.24685
948_s_at     4.88448     4.50453
949_s_at     4.83464     4.59238
950_at       4.54531     4.29197
951_at       2.98845     2.58530
952_at       2.60626     1.89240
```

[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

```
953_g_at     5.31042     4.89333
954_s_at     6.40030     6.06977
955_at       6.62752     6.34671
956_at       6.99861     6.70692
957_at       4.69390     4.34245
958_s_at     5.55759     5.19415
959_at       1.99939     1.66466
960_g_at     5.23385     4.94223
961_at       1.84130     1.62690
962_at       1.60305     1.55421
963_at       4.69636     4.18588
964_at       4.30356     4.07966
965_at       2.27711     1.33576
966_at       4.45459     4.06127
967_g_at     4.86522     4.59589
968_i_at     3.66416     3.57689
969_s_at     4.79956     4.55295
970_r_at     6.28942     6.17328
971_s_at     2.84888     2.46289
973_at       4.32935     4.10866
974_at       1.87040     2.29331
975_at       4.38042     4.08503
976_s_at     3.86166     3.11784
977_s_at     4.93245     4.56452
978_at       2.68011     2.59216
```

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. 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:

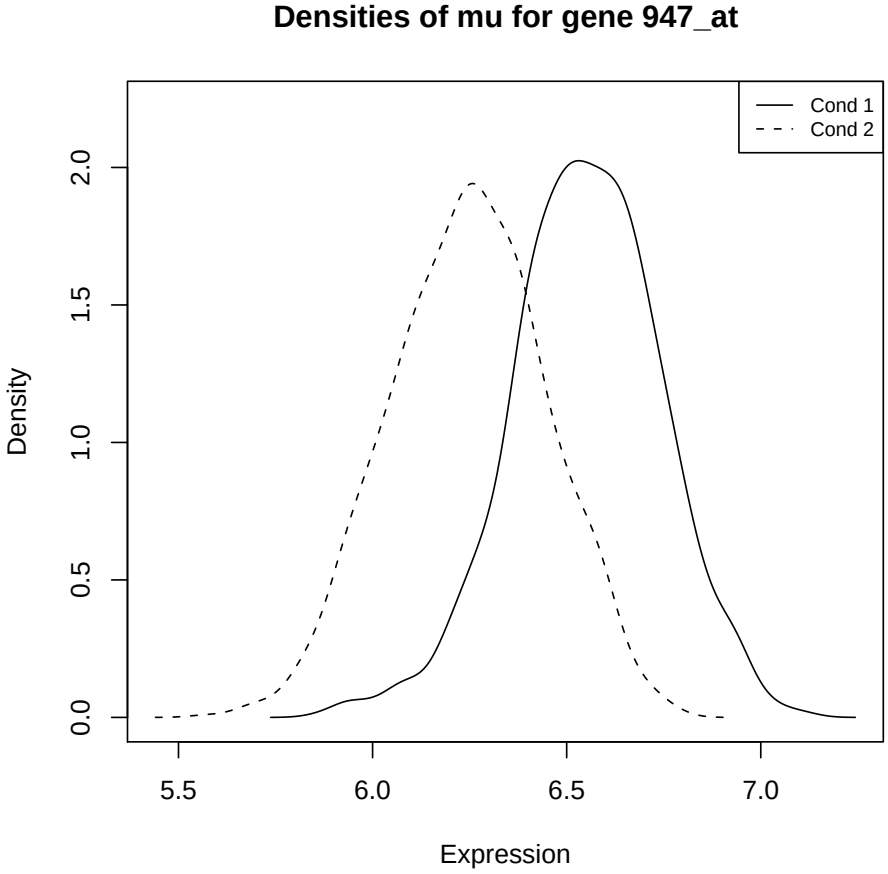
```
> bgxOutput <- readOutput.bgx("run.1")
```

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)
```

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

[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, ])
```

	Position	DiffExpression
956_at	19	35.857720
941_at	4	34.057708
AFFX-HSAC07/X00351_5_at	83	33.431479
955_at	18	32.120215
AFFX-HUMGAPDH/M33197_5_at	90	30.094049
954_s_at	17	29.230007
947_at	10	28.072969
AFFX-HUMGAPDH/M33197_M_at	92	26.088639
AFFX-HSAC07/X00351_M_at	85	22.047845
946_at	9	19.112386
AFFX-HUMGAPDH/M33197_3_at	88	17.709673
AFFX-hum_alu_at	87	16.605758

AFFX-BioDn-3_at	70	16.509066
953_g_at	16	16.160847
958_s_at	21	15.866340
AFFX-HUMISGF3A/M97935_3_at	94	14.627333
AFFX-HUMISGF3A/M97935_MB_at	97	13.385993
977_s_at	39	12.298497
982_at	44	12.020658
993_at	54	10.692664
948_s_at	11	10.648885
969_s_at	32	10.233128
AFFX-HSAC07/X00351_3_at	81	10.186931
960_g_at	23	10.179841
957_at	20	9.017495

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