

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 Bioconductor¹ 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* argument²; 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* argument³; 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	6.55138	6.26038
948_s_at	4.81587	4.51607
949_s_at	4.84355	4.63399
950_at	4.56655	4.31742
951_at	2.43563	2.72552
952_at	2.00411	2.53086

²Note 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	5.32531	4.90937
954_s_at	6.37378	6.07759
955_at	6.62420	6.35586
956_at	7.00169	6.71399
957_at	4.63236	4.33294
958_s_at	5.56037	5.18119
959_at	1.47410	1.40060
960_g_at	5.25870	4.96330
961_at	2.04249	1.34850
962_at	2.36443	1.57513
963_at	4.60603	4.34192
964_at	4.29928	4.09498
965_at	1.57227	2.06872
966_at	4.41798	4.10954
967_g_at	4.90560	4.61491
968_i_at	2.43029	3.71769
969_s_at	4.85067	4.55694
970_r_at	6.31518	6.17359
971_s_at	2.44516	2.49688
973_at	4.46834	4.12362
974_at	2.86450	2.29705
975_at	4.32882	4.10482
976_s_at	3.67115	2.81030
977_s_at	4.93549	4.60938
978_at	2.83836	2.19194

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 line⁴. 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 executable⁵, 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:

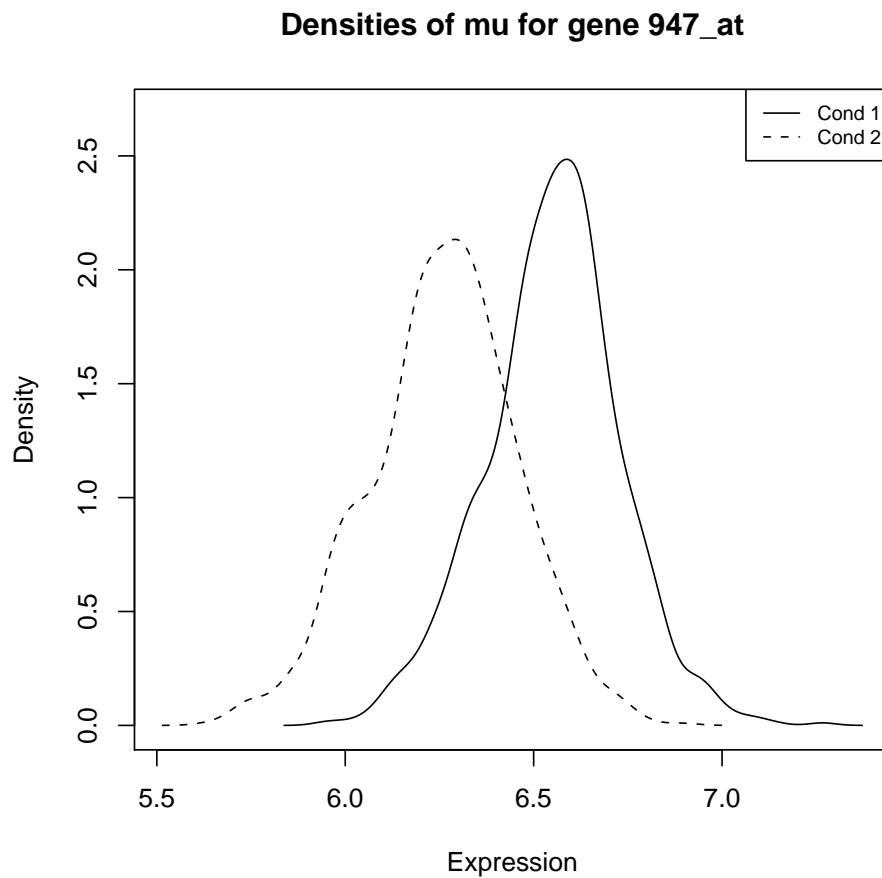
```
> bgxOutput <- readOutput.bgx("run.1")
```

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)
```

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

⁵Under 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
955_at	18	33.222281
AFFX-HSAC07/X00351_5_at	83	31.507561
AFFX-HUMGAPDH/M33197_5_at	90	29.928834
956_at	19	28.704045
941_at	4	27.843495
947_at	10	25.893534
AFFX-HSAC07/X00351_M_at	85	25.126291
AFFX-HUMGAPDH/M33197_M_at	92	24.585403
954_s_at	17	22.634570
953_g_at	16	22.067209
958_s_at	21	20.690656
AFFX-hum_alu_at	87	20.199556

AFFX-HUMGAPDH/M33197_3_at	88	19.762552
946_at	9	19.069500
AFFX-BioDn-3_at	70	16.881780
AFFX-HUMISGF3A/M97935_MB_at	97	13.474122
982_at	44	12.165461
977_s_at	39	12.136329
960_g_at	23	11.765490
942_at	5	11.391190
AFFX-HUMISGF3A/M97935_3_at	94	10.783033
AFFX-HUMISGF3A/M97935_MA_at	96	9.935485
969_s_at	32	8.037317
AFFX-HSAC07/X00351_3_at	81	8.016859
967_g_at	30	7.957880

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