# 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<sup>1</sup> 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()</pre>

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")

```
<sup>1</sup>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 Expression-Set 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<sup>2</sup>; 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<sup>3</sup>; 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")</pre>
```

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	6.55658	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

<sup>2</sup>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	5.28602	4.84581
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<sup>4</sup>. 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.

Once you have saved the input files, you should locate the binary, make sure it is executable<sup>5</sup>, 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:

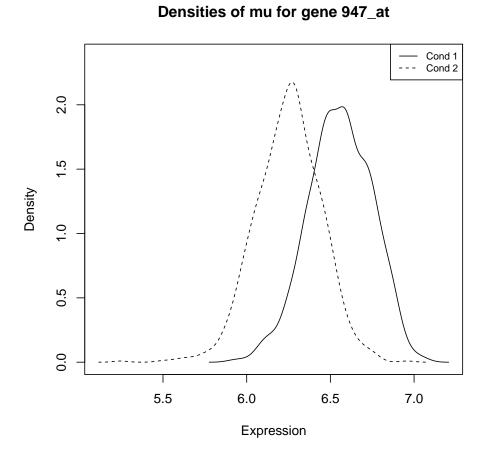
```
> bgxOutput <- readOutput.bgx("run.1")</pre>
```

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)
```

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

<sup>&</sup>lt;sup>5</sup>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)</pre>
```

```
> print(rankedGeneList[1:25, ])
```

	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.