# Textual description of affydata

### April 17, 2025

This is a simple *data package*. It provides an example dataset drawn from an actual Dilution experiment done by Gene Logic (http://www.genelogic.com/support/scientific-studies/). The full Dilution dataset is available on request (info@genelogic.com or +1-800-GENELOGIC). The help file ?Dilution describes the example dataset.

## 1 Normalization of Dilution Data

We start by loading the library and the data.

- > library(affydata)
- > data(Dilution)

This will create the Dilution object of class AffyBatch. As described in the affy vignette, print (or show) will display summary information. These objects represent data from one experiment. The AffyBatch class combines the information of various CEL files with a common CDF file. This class is designed to keep information of one experiment. The probe level data is contained in this object.

#### > Dilution

AffyBatch object size of arrays=640x640 features (38422 kb) cdf=HG\_U95Av2 (12625 affyids) number of samples=4 number of genes=12625 annotation=hgu95av2 notes=

The data in Dilution is a small sample of probe sets from 2 sets of duplicate arrays hybridized with different concentrations of the same RNA. This information is part of the AffyBatch and can be accessed with the phenoData and pData methods:

### > phenoData(Dilution)

An object of class 'AnnotatedDataFrame'

sampleNames: 20A 20B 10A 10B varLabels: liver sn19 scanner varMetadata: labelDescription

#### > pData(Dilution)

	liver	sn19	scanner
20A	20	0	1
20B	20	0	2
10A	10	0	1
10B	10	0	2

Various researchers have pointed out the need for normalization of Affymetrix arrays. Let's look at an example. The first two arrays in Dilution are technical replicates (same RNA), so the intensities obtained from these should be about the same. The second 2 are also replicates. The second arrays are hybridized to twice as much RNA so the intensities should be in general bigger. However, notice that the scanner effect is stronger than the RNA concentration effect.

#### > pData(Dilution) ##notice the scanner covariate

	liver	sn19	scanner
20A	20	0	1
20B	20	0	2
10A	10	0	1
10B	10	0	2

The boxplot method for the AffyBatch class, shown in Figure 1, shows this is the case.

The method boxplot can be used to show PM, MM or both intensities.

As discussed in the next section this plot shows that we need to normalize these arrays.

Figure 1 shows the need for normalization. For example arrays scanned using scanner 1 are globally larger than those scanned with 2.

Another way to see that normalization is needed is by looking at log ratio versus average log intensity (MVA) plots. The method mva.pairs will show all MVA plots of each pairwise comparison on the top right half and the interquartile range (IQR) of the log ratios on the bottom left half. For replicates and cases where most genes are not differentially expressed, we want the cloud of points to be around 0 and the IQR to be small.

The method normalize lets one normalize the data.

```
> par(mfrow=c(1,1))
> boxplot(Dilution,col=c(2,2,3,3))
```

## Small part of dilution study

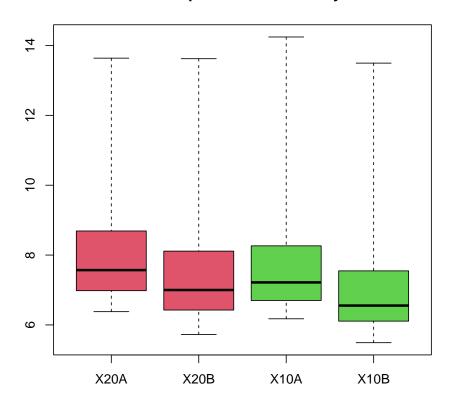


Figure 1: Boxplot of arrays in dilution data.

> gn <- sample(geneNames(Dilution),100) ##pick only a few genes
> pms <- pm(Dilution[,3:4], gn)
> mva.pairs(pms)

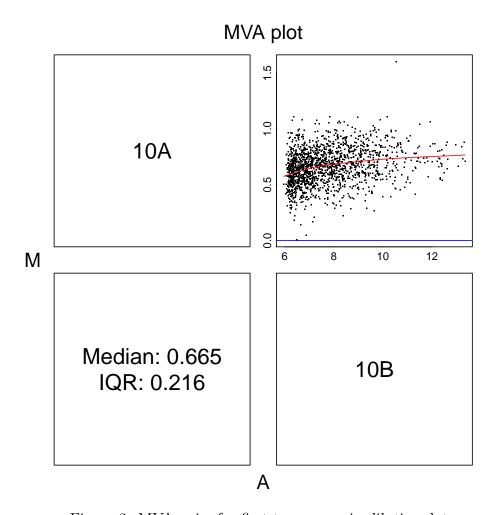


Figure 2: MVA pairs for first two arrays in dilution data

We normalize the two concentration groups separately. Notice the function merge permits us to put together two AffyBatch objects.

Various methods are available for normalization (see the help file). The default is quantile normalization. All the available methods are obtained using this function:

#### > normalize.methods(Dilution)

[1]	"constant"	"contrasts"	"invariantset"	"loess"
[5]	"methods"	"gspline"	"quantiles"	"quantiles.robust"

and can be called using the method argument of the normalize function.

Figures 1 and 1 show the boxplot and mva pairs plot after normalization. The normalization routine seems to correct the boxplots and mva plots.

> boxplot(normalized.Dilution, col=c(2,2,3,3), main="Normalized Arrays")

## **Normalized Arrays**

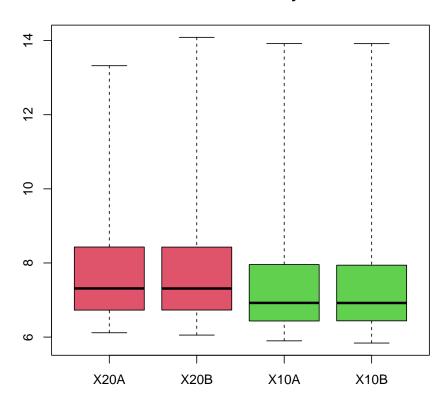


Figure 3: Boxplot of first arrays in normalized dilution data.

> pms <- pm(normalized.Dilution[, 3:4],gn)
> mva.pairs(pms)

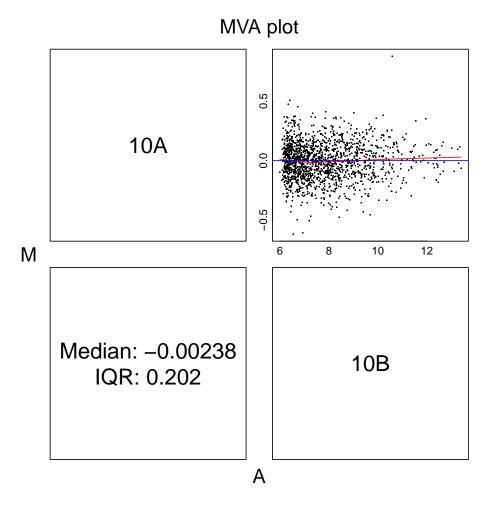


Figure 4: MVA pairs for first two replicate arrays in normalized dilution data