Extracting limma objects from limmaGUI files

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This vignette gives a short example showing how to extract limma data objects from files saved by limmaGUI. This could be used for advanced limma analysis by an expert user after some preliminary analysis with limmaGUI by someone unfamiliar with the command-line interface.
We will use a file `SwirlLinearModelComputed.lma` which has been saved from limmaGUI. It is available from:
`http://bioinf.wehi.edu.au/limmaGUI/Swirl/SwirlLinearModelComputed.lma`.
The `.lma` extension used by limmaGUI is simply a three-letter abbreviation of limma (Linear Models for Microarrays). This file is in fact a standard `.RData` file and can be loaded into any R session as described below.

```
> load("SwirlLinearModelComputed.lma")
```

Firstly, let's load the limma package, so that R knows how to display objects defined by limma classes (e.g. `RGList`).

```
> library(limma)
```

Now let's have a look at the R objects available to us:

```
> ls()
```

Now let's look at the RNA targets for this dataset:

```
> Targets
```

Now let's look at the first 30 lines of the genelist (Genepix Array List) for this dataset:

```
> gal[1:30,]
```

The raw red and green foreground and background intensities are stored the object `RG`. They can be viewed as follows:

```
> RG
```

Now if this `.lma` was saved by a recent version of limmaGUI (>=0.7.6), there should be an object available called `BCMethod` which contains the current background correction method. In old versions of limmaGUI, the only background correction method available was `"subtract"`, i.e. simply subtracting the background intensities from the foreground.

```
> BCMethod
```

Now let's see what MA objects are currently available to us. `MA.Available` is a list object with components `"Raw"` `"WithinArrays"`, `"BetweenArrays"` and `"Both"`, so for example `MA$Both` would be TRUE if there is an MA object which has been normalized both within arrays and between arrays. The four corresponding MA objects are called `MAraw`, `MAwithinArrays`, `MAbetweenArrays` and `MAboth`. Each one is initialised to an empty list, and if needed, it it overwritten with an appropriate MAList object.

```
> MA.Available
```

```
> MAraw
```

Now let's see how many parameterizations have been defined (i.e. how many design matrices).

```
> NumParameterizations
```

In this case, there is only one parameterization. Now let's have a look at the objects stored within this parameterization. The '1' in double square-brackets represents the first parameterization.

```
> names(ParameterizationList[[1]])
```

There is an object called designList, which is a list object containing the design matrix, and some information about how the user created that design matrix, in this case by requesting a comparison between `"Swirl"` and `"Wild Type"` using drop-down comboboxes, rather than manually entering the matrix numerically.

```
> ParameterizationList[[1]]$designList
```

Now let's look at the linear model fit object. Until version 0.7.7, limmaGUI used the old lm.series from limma rather than lmFit, so the fit object was a standard R list object, but from 0.7.7, lmFit is used so that the fit object is an object of class MArrayLM. This means that with a fit object obtained from a new limmaGUI analysis, typing `ParameterizationList[[1]]$fit` and pressing enter should display a summary of the data in the fit object, rather than all the data.

```
> ParameterizationList[[1]]$fit
```

Empirical bayes statistics can be obtained from the `"eb"` component of `ParameterizationList[[1]]`. Note that recent versions of limma encourage users to calculate empirical bayes statistics using `eBayes`, rather than `ebayes`, whereas at the time of writing limmaGUI still uses the old `ebayes` method, which produces a standard list object, meaning that typing `ParameterizationList[[1]]$eb` and pressing enter will display all the data in the list, rather than a summary. The components of the empirical bayes list object can be viewed as follows:

```
> names(ParameterizationList[[1]]$eb)
```

For example, the moderated t statistics can be obtained as follows:

```
> ParameterizationList[[1]]$eb$t
```

Other objects of interest include:

- `ParameterizationList[[1]]$WhetherToNormalizeWithinArrays`,
- `ParameterizationList[[1]]$WhetherToNormalizeBetweenArrays`,
- `ParameterizationList[[1]]$WithinArrayNormalizationMethod`,
- `ParameterizationList[[1]]$SpotTypesForLinearModel`, and
- `SpotTypes`.