affyPLM: Fitting Probe Level Models

Ben Bolstad

bmb@bmbolstad.com

http://bmbolstad.com

April 30, 2024

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1 Introduction

This majority of this document describes the fitPLM function. Other vignettes for affyPLM describe quality assessment tools and the threestep function for computing expression measures. After starting R, the package should be loaded using:

```r
> library(affyPLM)
> require(affydata)
> data(Dilution)  # an example dataset provided by the affydata package
> ##FIXME: drop this after Dilution is updated
> Dilution = updateObject(Dilution)
> options(width=36)
```

this will load affyPLM as well as the affy package and its dependencies. The Dilution dataset will serve as an example dataset for this document.

2 Fitting Probe Level Models

2.1 What is a Probe Level Model and What is a PLMset?

A probe level model (PLM) is a model that is fit to probe-intensity data. More specifically, it is where we fit a model with probe level and chip level parameters on a probeset by probeset basis. It is easy to arrange the probe-intensity data for a probeset so that the rows are probes and the columns are chips. In this case, our probe level parameters could be factor variable for each probe. The chip level parameters might be a factor variable with a level for each array, factor variables grouping the chips into treatment groups or perhaps some sort of covariate variable (pH, temperature, etc).

A PLMset is an R object that holds the results of a fitted probe level model. Among the items stored are parameter estimates and corresponding standard errors, weights and residuals.

2.2 Getting Started with the Default Model

The main function for fitting PLM is the function fitPLM. The easiest way to call the function is to call it by passing an AffyBatch object without any other arguments, this will fit a linear model with an effect estimated for each chip and an effect for each probe. This can be accomplished using:

```r
> Pset <- fitPLM(Dilution)
```
Once you have a fitted model stored in a \textit{PLMset} object the chip level parameter estimates and the corresponding standard errors can be examined using the accessor functions \texttt{coefs} and \texttt{se} respectively. For example, to examine the parameter estimates for the first 5 probesets and their corresponding standard error estimates use:

\begin{verbatim}
> coefs(Pset)[1:5,]

                20A    20B
100_g_at  7.485385  7.321158
1000_at   7.712294  7.589361
1001_at   5.403364  5.246518
1002_f_at 6.001354  5.842818
1003_s_at 6.682994  6.369082

10A    10B
100_g_at  7.421751  7.369140
1000_at   7.604202  7.553845
1001_at   5.273806  5.361613
1002_f_at 5.875315  5.901890
1003_s_at 6.497949  6.402645

> se(Pset)[1:5,]

                20A    20B
100_g_at  0.04237992 0.04176901
1000_at   0.03798884 0.03674159
1001_at   0.05850398 0.05889818
1002_f_at 0.06684381 0.06552410
1003_s_at 0.06114870 0.05960099

10A    10B
100_g_at  0.04498466 0.04278185
1000_at   0.03947771 0.03526104
1001_at   0.05917896 0.05606087
1002_f_at 0.06373685 0.06468718
1003_s_at 0.06161744 0.06088377
\end{verbatim}

Note that the default model is the RMA expression measure model. Specifically, the default model is

\[
\log_2 \text{PM}_{kij} = \beta_{kj} + \alpha_{ki} + \epsilon_{kij}
\]

where $\beta_{kj}$ is the log$_2$ gene expression value on array $j$ for probeset $k$ and $\alpha_{ki}$ are probe effects. Note that to make the model identifiable the constraint $\sum_{j=1}^{J} \alpha_{ki} = 0$ is used. Thus, for this default model, the parameter estimates given are gene expression values.
2.3 Getting Full Control over fitPLM

While the default model is very useful and simple to use, the fitPLM function also provides the user with a great deal of control. Specifically, the user has the ability to change the preprocessing, how the model is fitted and what output is returned.

2.3.1 Pre-processing

By default, the fitPLM function will preprocess the data using the RMA preprocessing steps. In particular, it uses the same background and normalization as the rma function of the affy package. It is possible to turn off either of these preprocessing steps by specifying that background and/or normalize are FALSE in the call to fitPLM. The arguments background.method and normalize.method can be used to control which pre-processing methods are used. The same preprocessing methods, as described in the threestep vignette, may be used with the fitPLM function.

2.3.2 Controlling what is Returned in the PLMset

The PLMset that the fitPLM function returns contains a number of different quantities, some of which are always returned such as parameter estimates and standard error estimates and others which are more optional. The user can control whether weights, residuals, variance-covariance matrices and residual standard deviations are returned. By default, all of these items are returned, but in certain situations a user might find it useful to exclude certain items to save memory. Control is via the output.param argument which should be provided as a list. The default settings can be seen by typing

> verify.output.param()

$weights
[1] TRUE

$residuals
[1] TRUE

$varcov
[1] "none"

$resid.SE
[1] TRUE

Control of whether weights, residuals and residual standard deviations are returned is via logical variables. There are three options varcov = "none", varcov = "chiplevel" and varcov = "all" for variance covariance matrices. These correspond to, not returning any variance estimates, only the portion of the variance covariance matrix related to
the chiplevel parameters or the entire variance-covariance matrix respectively. When each probeset has a large number of probes (or there are large numbers of parameters in the model) the last option will return many large variance-covariance matrices. The following code returns a PLMset with no weights or residuals stored:

\[
Pset <- \text{fitPLM(Dilution, output.param=list(residuals=FALSE, weights=FALSE))}
\]

2.3.3 Controlling how the model is fit

fitPLM implements iteratively re-weighted least squares M-estimation regression. Control over how fitPLM carries out the model fitting procedure is given by the model.param argument. This value of this parameter should be a list of settings. In particular, these settings are the following:

- **trans.fn** which controls how the response variable is transformed. This value should be a string. By default trans.fn="log2", but other possible options include: "loge" or "ln" to use the natural logarithm, "log10" for logarithm base 10, "sqrt" for square root and "cuberoot" to use a cubic root transformation.

- **se.type** which controls how the variance-covariance matrix is estimated in the M-estimation procedure. Possible values are 1, 2, 3 or 4. See the Appendix for more details.

- **psi.type** is a string which selects how the weights are computed in the robust regression. By default psi.type="Huber". Other possible options include "fair", "Cauchy", "Geman-McClure", "Welsch", "Tukey", and "Andrews". More details can be found in the Appendix.

- **psi.k** is a numerical tuning constant used by psi.type. The default values are dependent on the option chosen by psi.type. More details can be found in the Appendix.

- **max.its** controls the maximum number of iterations of IRLS that will be used in the model fitting procedure. By default max.its=20. Note, that this many iterations may not be needed if convergence occurs.

- **init.method** controls how the initial parameter estimates are derived. By default init.method="ls" ordinary least squares is used although "Huber" is also a possibility.

- **weights.chip** are input weights for each chip in the dataset. This parameter should be a vector of length number of arrays in the dataset. By default, every chip is given equal weight.
• weights.probe are input weights for each probe in the dataset. This parameter should be a vector of length number of probes in dataset (this length depends on the response variable in the model). By default, every probe has equal weight.

As an example, we use model.param to control the fitting procedure so that it is fit as a standard linear regression model (ie without robustness). This is accomplished by:

```r
> Pset <- fitPLM(Dilution, model.param=list(max.its=0))
```

### 2.4 Specifying models in fitPLM

Although the default model is very useful, it is by no means the only model that can be fitted using fitPLM. In this section we describe many, but certainly not all the different types of models which may be fitted. In the example code presented here we will use the subset argument to restrict model fitting to the first 100 probesets for speed. In any real analysis model fitting would be carried out to all probesets on a particular array type.
2.4.1 Notation

$i$  Index for probes $i = 1, \ldots, I_k$

$j$  Index for arrays $j = 1, \ldots, J$

$k$  Index for probeset $k = 1, \ldots, K$

$l$  Index for probe type $l = 1, 2$ where $1$ is PM and $2$ is MM.

$m$  Index for level of primary treatment factor variable $m = 1, \ldots, M$

$\alpha_{ki}$  probe effect parameter for probe $i$

Included in the model by using probes

$\alpha_{kim}$  probe effect parameter for probe $i$ estimated only for arrays where

primary treatment factor variable is level $m$

Included in the model by using treatment:probes

$\alpha_{kil}$  probe effect parameter for probe $i$ estimated only for probes of

type $l$

Included in the model by using probe.type:probes

$\alpha_{kilm}$  probe effect parameter for probe $i$ estimated only for probes of

type $l$ where primary treatment factor variable is level $m$

Included in the model by using treatment:probe.type:probes

$\beta_{kj}$  array (chip) effect.

Included in the model by using samples

$\phi_{kl}$  probe-type effect

Included in the model by using probe.types

$\phi_{kim}$  probe-type effect for probe type $l$ estimated only for arrays where

primary treatment factor variable is level $m$

Included in the model by using treatment:probe.types

$\phi_{klj}$  probe-type effect for probe type $l$ estimated only for array $j$

Included in the model by using samples:probe.types

$\theta$  a vector of chip-level parameters

$\mu_k$  an intercept parameter

$\gamma_k$  a slope parameter

$y_{kijl}$  a processed probe-intensity. Typically on log$_2$ scale.

$\epsilon_{kijl}$  an error term

$x_j$  measurements of chip-level factor and covariate variables for chip $j$

In the model descriptions below we will use treatment, trt.cov for these terms. In practice these would be replaced with names of variables in the current R environment or the phenoData of the supplied AffyBatch.

Since we are focusing on models that are fitted in a probeset by probeset manner for brevity the subscript $k$ will be omitted from further discussion. Note the terms probes, samples and probe.types are considered reserved words when specifying a model to fitPLM.
2.4.2 RMA style PLM

These are variations of the RMA model each consisting of models with chip and probe-effects. The first, \( PM \sim -1 + \text{samples} + \text{probes} \), is the default model used when no model is specified in the fitPLM call.

<table>
<thead>
<tr>
<th>Model</th>
<th>fitPLM syntax</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y_{ij1} = \beta_j + \alpha_i + \epsilon_{ij} )</td>
<td>( PM \sim -1 + \text{samples} + \text{probes} )</td>
</tr>
<tr>
<td>( y_{ij1} = \mu + \beta_j + \alpha_i + \epsilon_{ij} )</td>
<td>( PM \sim \text{samples} + \text{probes} )</td>
</tr>
<tr>
<td>( y_{ij1} = \beta_j + \epsilon_{ij} )</td>
<td>( PM \sim -1 + \text{samples} )</td>
</tr>
<tr>
<td>( y_{ij1} = \mu + \beta_j + \epsilon_{ij} )</td>
<td>( PM \sim \text{samples} )</td>
</tr>
<tr>
<td>( y_{ij2} = \beta_j + \alpha_i + \epsilon_{ij} )</td>
<td>( MM \sim -1 + \text{samples} + \text{probes} )</td>
</tr>
<tr>
<td>( y_{ij2} = \mu + \beta_j + \alpha_i + \epsilon_{ij} )</td>
<td>( MM \sim \text{samples} + \text{probes} )</td>
</tr>
<tr>
<td>( y_{ij2} = \beta_j + \epsilon_{ij} )</td>
<td>( MM \sim -1 + \text{samples} )</td>
</tr>
<tr>
<td>( y_{ij2} = \mu + \beta_j + \epsilon_{ij} )</td>
<td>( MM \sim \text{samples} )</td>
</tr>
</tbody>
</table>

2.4.3 PLM with chip-level factor and covariate variables

These models use treatment variables as an alternative to sample effects for the chip level factors.

<table>
<thead>
<tr>
<th>Model</th>
<th>fitPLM syntax</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y_{ij1} = x_j^T \theta + \alpha_i + \epsilon_{ij} )</td>
<td>( PM \sim -1 + \text{treatment} + \text{trt.cov} + \text{probes} )</td>
</tr>
<tr>
<td>( y_{ij1} = x_j^T \theta + \epsilon_{ij} )</td>
<td>( PM \sim -1 + \text{treatment} + \text{trt.cov} )</td>
</tr>
<tr>
<td>( y_{ij2} = x_j^T \theta + \alpha_i + \epsilon_{ij} )</td>
<td>( MM \sim -1 + \text{treatment} + \text{trt.cov} + \text{probes} )</td>
</tr>
<tr>
<td>( y_{ij2} = x_j^T \theta + \epsilon_{ij} )</td>
<td>( MM \sim -1 + \text{treatment} + \text{trt.cov} )</td>
</tr>
</tbody>
</table>

For example to fit, a model with effects for both liver tissue concentration and scanner along with probe effects with MM as the response variable to the first 100 probesets of the Dilution dataset the following code would be used:

\[
> \text{Pset <- fitPLM(Dilution, MM \sim -1 + \text{liver} + \text{scanner} + \text{probes},subset = geneNames(Dilution)[1:100])}
\]

Examining the fitted chip-level parameter estimates for the first probeset via:

\[
> \text{coefs(Pset)[1,]}
\]

\[
\text{liver_10 liver_20 scanner_2} \\
6.89473332 6.84583436 -0.02337941
\]

shows that the treatment effect for scanner was constrained to make the model identifiable. fitPLM always leaves the first factor variable unconstrained if there is no intercept term. All other chip level factor variables are constrained. The parameter estimates for the probe effects can be examined as follows:

\[
> \text{coefs.probe(Pset)[1]}
\]
To make a treatment variable as a covariate rather than a factor variable the `variable.type` argument may be used. For example, to fit a model with the logarithm of liver concentration treated as a covariate we could do the following:

```r
> logliver <- log2(c(20,20,10,10))
> Pset <- fitPLM(Dilution,model=PM~1+probes+logliver+scanner, variable.type=c(logliver="covariate"),subset = geneNames(Dilution)[1:100])
```

Warning: No default variable type so assuming 'factor'

```r
> coefs(Pset)[1,]
```

```r
logliver  scanner_1  scanner_2
-0.01340544  7.44008342  7.37540612
```

### 2.4.4 Probe intensity covariate PLM

This class of models allows the inclusion of PM or MM probe intensities as covariate variables in the model. Note that the fitting methods currently used by `fitPLM` are robust, but not resistant (i.e., outliers in the response variable are dealt with, but outliers in explanatory variables are not).
To fit a model with an intercept term, MM covariate variable, sample and probe effects use the following code:

```r
>Pset <- fitPLM(Dilution, PM ~ MM + samples + probes, subset = geneNames(Dilution)[1:10])
```

We can examine the various parameter estimates for the model fit to the first probeset using:

```r
> coefs(Pset)[1,]

<table>
<thead>
<tr>
<th></th>
<th>20B</th>
<th>10A</th>
<th>10B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.10071600</td>
<td>-0.05355776</td>
<td>-0.07452209</td>
</tr>
</tbody>
</table>

> coefs.const(Pset)[1,]

<table>
<thead>
<tr>
<th>Intercept</th>
<th>MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0192030</td>
<td>0.2032069</td>
</tr>
</tbody>
</table>

> coefs.probe(Pset)[1]

```

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>probe_1</td>
<td>-0.8364048</td>
</tr>
<tr>
<td>probe_2</td>
<td>1.1502090</td>
</tr>
<tr>
<td>probe_3</td>
<td>1.6239088</td>
</tr>
<tr>
<td>probe_4</td>
<td>-1.8408843</td>
</tr>
<tr>
<td>probe_5</td>
<td>0.7334505</td>
</tr>
<tr>
<td>probe_6</td>
<td>-1.9505552</td>
</tr>
<tr>
<td>probe_7</td>
<td>0.7185392</td>
</tr>
<tr>
<td>probe_8</td>
<td>0.6929887</td>
</tr>
<tr>
<td>probe_9</td>
<td>-0.5038822</td>
</tr>
<tr>
<td>probe_10</td>
<td>-0.6445614</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>10A</th>
<th>10B</th>
</tr>
</thead>
<tbody>
<tr>
<td>probe_1</td>
<td>20B</td>
<td>-0.8364048</td>
</tr>
<tr>
<td>probe_2</td>
<td>10A</td>
<td>1.1502090</td>
</tr>
<tr>
<td>probe_3</td>
<td>10B</td>
<td>1.6239088</td>
</tr>
<tr>
<td>probe_4</td>
<td>10B</td>
<td>-1.8408843</td>
</tr>
<tr>
<td>probe_5</td>
<td>10B</td>
<td>0.7334505</td>
</tr>
<tr>
<td>probe_6</td>
<td>10B</td>
<td>-1.9505552</td>
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<td>10B</td>
<td>0.7185392</td>
</tr>
<tr>
<td>probe_8</td>
<td>10B</td>
<td>0.6929887</td>
</tr>
<tr>
<td>probe_9</td>
<td>10B</td>
<td>-0.5038822</td>
</tr>
<tr>
<td>probe_10</td>
<td>10B</td>
<td>-0.6445614</td>
</tr>
</tbody>
</table>
As can be seen by this example code intercept and covariate parameters are accessed using `coefs.const`.

### 2.4.5 PLM with both probe types as response variables

It is possible to fit a model that uses both PM and MM intensities as the response variable. This is done by specifying `PMMM` as the response term in the model. When both PM and MM intensities are used as the response, there is a special reserved term `probe.type` which may (optionally) be used as part of the model specification. This term designates that a probe type effect (ie whether PM or MM) should be included in the model.

<table>
<thead>
<tr>
<th>Model</th>
<th>fitPLM syntax</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y_{ijl} = \beta_j + \phi_j + \alpha_i + \epsilon_{ijl} )</td>
<td><code>PMMM ~ -1 + samples + probe.type + probes</code></td>
</tr>
<tr>
<td>( y_{ijl} = \mu + \beta_j + \phi_j + \alpha_i + \epsilon_{ijl} )</td>
<td><code>PMMM ~ samples + probe.type + probes</code></td>
</tr>
<tr>
<td>( y_{ijl} = \beta_j + \phi_j + \epsilon_{ijl} )</td>
<td><code>PMMM ~ -1 + samples + probe.type</code></td>
</tr>
<tr>
<td>( y_{ijl} = \mu + \beta_j + \phi_j + \epsilon_{ijl} )</td>
<td><code>PMMM ~ samples + probe.type</code></td>
</tr>
<tr>
<td>( y_{ijl} = x_j^T \theta + \phi_j + \epsilon_{ijl} )</td>
<td><code>PMMM ~ treatment + trt.cov + probe.type + probes</code></td>
</tr>
<tr>
<td>( y_{ijl} = x_j^T \theta + \phi_j + \alpha_i + \epsilon_{ijl} )</td>
<td><code>PMMM ~ treatment + trt.cov + probe.type + probes</code></td>
</tr>
<tr>
<td>( y_{ijl} = x_j^T \theta + \phi_j + \epsilon_{ijl} )</td>
<td><code>PMMM ~ treatment + trt.cov + probe.type</code></td>
</tr>
</tbody>
</table>

For example to fit such a model with factor variables for liver RNA concentration, probe type and probe effects use:

```r
> Pset <- fitPLM(Dilution, PMMM ~ liver + probe.type + probes, subset = geneNames(Dilution)[1:100])
```

Examining the parameter estimates:

```r
> coefs(Pset)[1,]
[1] -0.05141184

> coefs.const(Pset)[1,]

    Intercept probe.type_MM
    7.3357755  -0.4446907

> coefs.probe(Pset)[1]
```
Overall
probe_1 -1.3181940
probe_2 1.0521928
probe_3 2.2619856
probe_4 -0.9595112
probe_5 0.5156337
probe_6 -1.7679152
probe_7 3.4232431
probe_8 0.5271538
probe_9 -1.1465813
probe_10 -1.1015433
probe_11 0.9862332
probe_12 0.4896713
probe_13 -1.5278630
probe_14 1.3515933
probe_15 0.1257186

shows that probe type estimates are also accessed by using \texttt{coefs.const}.

### 2.4.6 PLM with probe-effects estimated within levels of a chip-level factor variable

It is also possible to estimate separate probe-effects for each level of a chip-level factor variable.

<table>
<thead>
<tr>
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<th>fitPLM syntax</th>
</tr>
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<tbody>
<tr>
<td>$y_{ij1} = x_j^T \theta + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PM $\sim$ treatment:probes + treatment + trt.cov</td>
</tr>
<tr>
<td>$y_{ij1} = y_{ij2} \gamma + x_j^T \theta + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PM $\sim$ MM + treatment + treatment:probes + trt.cov</td>
</tr>
<tr>
<td>$y_{ij1} = x_j^T \theta + \phi_j + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PMMM $\sim$ treatment + trt.cov + treatment:probes</td>
</tr>
</tbody>
</table>

Fitting such a model with probe effects estimated within the levels of the liver variable is done with:

```r
Pset <- fitPLM(Dilution, PM ~ -1 + liver + liver:probes,subset = geneNames(Dilution)[1:100])
```

Examining the estimated probe-effects for the first probeset can be done via:

```r
coefs.probe(Pset)[1]
```

shows that probe type estimates are also accessed by using \texttt{coefs.const}.

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<td>PM $\sim$ treatment:probes + treatment + trt.cov</td>
</tr>
<tr>
<td>$y_{ij1} = y_{ij2} \gamma + x_j^T \theta + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PM $\sim$ MM + treatment + treatment:probes + trt.cov</td>
</tr>
<tr>
<td>$y_{ij1} = x_j^T \theta + \phi_j + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PMMM $\sim$ treatment + trt.cov + treatment:probes</td>
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```r
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<td>PM $\sim$ treatment:probes + treatment + trt.cov</td>
</tr>
<tr>
<td>$y_{ij1} = y_{ij2} \gamma + x_j^T \theta + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PM $\sim$ MM + treatment + treatment:probes + trt.cov</td>
</tr>
<tr>
<td>$y_{ij1} = x_j^T \theta + \phi_j + \alpha_{im} + \epsilon_{ij1}$</td>
<td>PMMM $\sim$ treatment + trt.cov + treatment:probes</td>
</tr>
</tbody>
</table>

Fitting such a model with probe effects estimated within the levels of the liver variable is done with:

```r
Pset <- fitPLM(Dilution, PM ~ -1 + liver + liver:probes,subset = geneNames(Dilution))
```

Examining the estimated probe-effects for the first probeset can be done via:

```r
coefs.probe(Pset)[1]
```
probe_4  -1.9643798 -1.6998292
probe_5   0.6912122  0.8848448
probe_6  -2.0584672 -2.4959521
probe_7   2.5266458  2.6174269
probe_8   0.5508511  0.9657275
probe_9  -0.8175456 -0.8471498
probe_10  -0.6359297 -1.0412153
probe_11  0.5274181  0.4060750
probe_12  0.7551887  0.8190649
probe_13 -1.5205326 -1.1813253
probe_14  1.1776476  1.0345574
probe_15  1.5904612  1.6949012

2.4.7 PLM with probe-effect estimated within probe.type

Probe effects can also be estimated within probe type or within probe type for each level of the primary treatment factor variable.

\[
\begin{align*}
y_{ijl} &= x_j^T \theta + \alpha_{il} + \epsilon_{ijl} & \text{PMMM} \sim \text{treatment + trt.cov + probe.type:probes} \\
y_{ijl} &= x_j^T \theta + \alpha_{ilm} + \epsilon_{ijl} & \text{PMMM} \sim \text{treatment + trt.cov + treatment:probe.type:probes}
\end{align*}
\]

As an example, use the following code to fit such models and then examine the possible

\[
\begin{align*}
> \text{Pset} &< - \text{fitPLM(Dilution, PMMM -1 + liver + probe.type:probes,subset = geneNames(Dilution))} \\
> \text{coefs.probe(Pset)[1]}
\end{align*}
\]

\[
\begin{align*}
> \text{Pset} &< - \text{fitPLM(Dilution, PMMM -1 + liver + liver:probe.type:probes,subset = geneNames(Dilution))} \\
> \text{coefs.probe(Pset)[1]}
\end{align*}
\]

2.4.8 PLM without chip level effects

It is possible to fit models which do not have any chip-level variables at all. If this is the case, then the probe type effect takes precedence over any probe effects in the model. That is it will be unconstrained.

\[
\begin{align*}
y_{ijl} &= \alpha_i + \phi_{jl} + \epsilon_{ijl} & \text{PMMM} \sim -1 + \text{probe.type + probes} \\
y_{ijl} &= \mu + \phi_{jl} + \alpha_i + \epsilon_{ijl} & \text{PMMM} \sim \text{probe.type + probes} \\
y_{ijl} &= \phi_t + \alpha_{im} + \epsilon_{ijl} & \text{PMMM} \sim -1 + \text{probe.type + treatment:probes} \\
y_{ijl} &= \mu + \phi_t + \alpha_{im} + \epsilon_{ijl} & \text{PMMM} \sim \text{probe.type + treatment:probes} \\
y_{ijl} &= \mu + \phi_{im} + \alpha_{im} + \epsilon_{ijl} & \text{PMMM} \sim \text{samples:probe.type + treatment:probes} \\
y_{ijl} &= \mu + \phi_{im} + \alpha_{im} + \epsilon_{ijl} & \text{PMMM} \sim \text{treatment:probe.type + treatment:probes}
\end{align*}
\]
2.4.9 PLM with only probe-effects

It is also possible to fit models where only probe effects alone are estimated.

<table>
<thead>
<tr>
<th>Model</th>
<th>fitPLM syntax</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y_{ij1} = \alpha_i + \epsilon_{ij1}$</td>
<td>PM ~ -1 + probes</td>
</tr>
<tr>
<td>$y_{ij1} = \mu + \alpha_i + \epsilon_{ij1}$</td>
<td>PM ~ probes</td>
</tr>
<tr>
<td>$y_{ij1} = \alpha_{im} + \epsilon_{ij1}$</td>
<td>PM ~ -1 + treatment:probes</td>
</tr>
<tr>
<td>$y_{ij1} = \mu + (\theta \alpha)<em>{mj} + \epsilon</em>{ij1}$</td>
<td>PM ~ treatment:probes</td>
</tr>
<tr>
<td>$y_{ij2} = \alpha_i + \epsilon_{ij2}$</td>
<td>MM ~ -1 + probes</td>
</tr>
<tr>
<td>$y_{ij2} = \mu + \alpha_i + \epsilon_{ij2}$</td>
<td>MM ~ probes</td>
</tr>
<tr>
<td>$y_{ij2} = \alpha_{im} + \epsilon_{ij2}$</td>
<td>MM ~ -1 + treatment:probes</td>
</tr>
<tr>
<td>$y_{ij2} = \mu + \alpha_{im} + \epsilon_{ij2}$</td>
<td>MM ~ treatment:probes</td>
</tr>
<tr>
<td>$y_{ijkl} = \alpha_i + \epsilon_{ijkl}$</td>
<td>PMMM ~ -1 + probes</td>
</tr>
<tr>
<td>$y_{ijkl} = \mu + \alpha_i + \epsilon_{ijkl}$</td>
<td>PMMM ~ probes</td>
</tr>
<tr>
<td>$y_{ijkl} = \alpha_{im} + \epsilon_{ijkl}$</td>
<td>PMMM ~ -1 + treatment:probes</td>
</tr>
<tr>
<td>$y_{ijkl} = \mu + \alpha_{im} + \epsilon_{ijkl}$</td>
<td>PMMM ~ treatment:probes</td>
</tr>
</tbody>
</table>

2.4.10 Constraints

These are the constraints that will be imposed to make the models identifiable (when needed):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Constraints</th>
<th>Default</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_i$</td>
<td>$\sum_{j=0}^{l} \beta_i = 0$ or $\beta_1 = 0$</td>
<td>$\beta_1 = 0$</td>
</tr>
<tr>
<td>$\phi_l$</td>
<td>$\sum_{j=1}^{l} \phi_l = 0$ or $\phi_1 = 0$</td>
<td>$\phi_1 = 0$</td>
</tr>
<tr>
<td>$\phi_{ij}$</td>
<td>$\sum_{j=1}^{l} \phi_{ij} = 0$ or $\phi_{1j} = 0$</td>
<td>$\phi_{1j} = 0$</td>
</tr>
<tr>
<td>$\phi_{lm}$</td>
<td>$\sum_{l=1}^{j} \phi_{lm} = 0$ or $\phi_{1m} = 0$</td>
<td>$\phi_{1m} = 0$</td>
</tr>
<tr>
<td>$\alpha_i$</td>
<td>$\sum_{j=0}^{l} \alpha_i = 0$ or $\alpha_1 = 0$</td>
<td>$\sum_{j=0}^{l} \alpha_i = 0$</td>
</tr>
<tr>
<td>$\alpha_{im}$</td>
<td>$\sum_{j=0}^{l} \alpha_{im} = 0$ or $\alpha_{1m} = 0$</td>
<td>$\sum_{j=0}^{l} \alpha_{im} = 0$</td>
</tr>
<tr>
<td>$\alpha_{il}$</td>
<td>$\sum_{j=0}^{l} \alpha_{il} = 0$ or $\alpha_{1l} = 0$</td>
<td>$\sum_{j=0}^{l} \alpha_{il} = 0$</td>
</tr>
<tr>
<td>$\alpha_{ilm}$</td>
<td>$\sum_{j=0}^{l} \alpha_{ilm} = 0$ or $\alpha_{1lm} = 0$</td>
<td>$\sum_{j=0}^{l} \alpha_{ilm} = 0$</td>
</tr>
</tbody>
</table>

In general, there is a general hierarchy by which items are left unconstrained:

intercept > treatment > sample > probe.type > probes

the highest term in this hierarchy that is in a particular model is always left unconstrained, everything else will be constrained. So for example a model containing probe.type and probe effects will have the probe.type effects unconstrained and the probe effects constrained.

Constraints are controlled using the constraint.type argument which is a vector with named items should be either "contr.sum" or "contr.treatment". The names for this vector should be names of items in the model.

> data(Dilution)
> ##FIXME: remove next line
> Dilution = updateObject(Dilution)
> Pset <- fitPLM(Dilution, model = PM ~ probes + samples,constraint.type=c(samples="contr.sum"),
>
> Warning: No default constraint specified. Assuming 'contr.treatment'.
>
> coefs.const(Pset)[1:2]
>
> [1] 7.355977 7.576212
>
> coefs(Pset)[1:2,]
>
>          20A      20B 100_g_at 0.03476993 -0.045356385 1000_at 0.03496940 0.008864674 10A 100_g_at 0.028454952 1000_at -0.001171587

3 How long will it take to run the model fitting procedures?

It may take considerable time to run the fitPLM function. The length of time it is going to take to run the model fitting procedure will depend on a number of factors including:

1. CPU speed
2. Memory size of the machine (RAM and VM)
3. Array type
4. Number of arrays
5. Number of parameters in model

It is recommended that you run the fitPLM function only on machines with large amounts of RAM. If you have a large number of arrays the number of parameters in your model will have the greatest effect on runtime.
4 Dealing with the PLMset object

As previously mentioned, the results of a call to fitPLM are stored in a PLMset object. There are a number of accessor functions that can be used to access values stored in a PLMset including:

- `coefs` and `se`: access chip-level factor/covariate parameter and standard error estimates.
- `coefs.probe` and `se.probe`: access probe effect parameter and standard error estimates.
- `coefs.const` and `se.const`: access intercept, MM covariate and probe type effect parameter and standard error estimates.
- `weights`: access final weights from M-estimation procedure. Note that you may optionally supply a vector of probeset names as a second parameter to get weights for only a subset of probes.
- `resids`: access residuals. Note that you may optionally supply a vector of probeset names as a second parameter to get residuals for only a subset of probes.
- `varcov`: access variance matrices.

A M-estimation: How fitPLM fits models

Suppose we wish to fit the following model

\[ y_i = f(x_i, \theta) + \epsilon_i \]  

(1)

where \( y_i \) is a response variable, \( x_i \) is a vector of explanatory variables, and \( \theta \) is a vector of parameters to be estimated. An estimator of \( \theta \) is given by

\[ \min_{\theta} \sum_{i=1}^{N} (y_i - f(x_i, \theta))^2 \]  

(2)

which is the known least squares estimator. In some situations, outliers in the response variable can have significant effect on the estimates of the parameters. To deal with potential problem we need a robust method of fitting the model. One such method is known as M-estimation. An M-estimator for this regression, taking into account scale, is the solution of

\[ \min_{\theta} \sum_{i=1}^{N} \rho \left( \frac{y_i - f(x_i, \theta)}{s} \right) \]  

(3)
where \( \rho \) is a suitable function. Reasonable properties for \( \rho \) include symmetry \( \rho(x) = \rho(-x) \), a minimum at \( \rho(0) = 0 \), positive \( \rho(x) \geq 0 \ \forall x \) and increasing as the absolute value of \( x \) increases, i.e. \( \rho(x_i) \geq \rho(x_j) \) if \( |x_i| > |x_j| \). Furthermore, there is the need to estimate \( s \), where \( s \) is a scale estimate. One approach is to estimate both \( s \) and \( \theta \) using a system of equations. The approach that we use is to estimate \( s \) using the median absolute deviation (MAD) which provides a robust estimate of scale. The above equation leads to solving

\[
\sum_{i=1}^{N} \psi \left( \frac{y_i - f(x_i, \theta)}{s} \right) = 0. \tag{4}
\]

where \( \psi \) is the derivative of \( \rho \). Note that strictly speaking, for robustness, \( \psi \) should be bounded. Now define \( r_i = \frac{y_i - f(x_i, \theta)}{s} \) and a weight function \( w(r_i) = \frac{\psi(r_i)}{r_i} \). Then the previous equation can be rewritten as

\[
\sum_{i=1}^{N} w(r_i) r_i = 0 \tag{5}
\]

which is the same as the set of equations that would be obtained if we were solving the iteratively re-weighted least squares problem

\[
\min \sum_{i} w(r_i^{(n-1)}) r_i^{(n)}^2 \tag{6}
\]

where the superscript \( (n) \) represents the iteration number. More details about M-estimation can be found in [Huber (1981)]. Tables 1 and 2 describe the various different weight functions and associated default constants provided by fitPLM.

**B Variance Matrix and Standard error estimates for fitPLM**

[Huber (1981)] gives three forms of asymptotic estimators for the variance-covariance matrix of parameter estimates \( \hat{b} \).

\[
\kappa^2 \frac{\sum \psi^2/(n-p)}{(\sum \psi^2/n)^2} (X^T X)^{-1} \tag{7}
\]

\[
\kappa^2 \frac{\sum \psi^2/(n-p)}{\sum \psi^2/n} V^{-1} \tag{8}
\]

\[
\frac{1}{\kappa} \frac{\sum \psi^2}{n-p} V^{-1} (X^T X) V^{-1} \tag{9}
\]

where

\[
\kappa = 1 + \frac{p}{n} \frac{\text{Var}(\psi')}{E\psi'} \tag{10}
\]
### Table 1: \( \rho, \psi \) and weight functions for some common M-estimators.

<table>
<thead>
<tr>
<th>Method</th>
<th>( \rho(x) )</th>
<th>( \psi(x) )</th>
<th>( w(x) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huber</td>
<td>( \begin{cases} x^2/2 &amp; \text{if }</td>
<td>x</td>
<td>\leq k \ k(</td>
</tr>
<tr>
<td>fair</td>
<td>( \frac{c^2}{2} \left( \frac{</td>
<td>x</td>
<td>}{c} - \log\left(1 + \frac{</td>
</tr>
<tr>
<td>Cauchy</td>
<td>( \frac{c^2}{2} \log\left(1 + (x/c)^2\right) )</td>
<td>( \frac{x}{1 + (x/c)^2} )</td>
<td>( \frac{1}{1 + (x/c)^2} )</td>
</tr>
<tr>
<td>Geman-McClure</td>
<td>( \frac{x^2/2}{1 + x^2} )</td>
<td>( \frac{x}{(1 + x^2)^2} )</td>
<td>( \frac{1}{(1 + x^2)^2} )</td>
</tr>
<tr>
<td>Welsch</td>
<td>( \frac{c^2}{2} \left( 1 - \exp\left(-\left(\frac{x}{c}\right)^2\right) \right) )</td>
<td>( x \exp\left(-\left(\frac{x}{c}\right)^2\right) )</td>
<td>( \exp\left(-\left(\frac{x}{c}\right)^2\right) )</td>
</tr>
<tr>
<td>Tukey</td>
<td>( \begin{cases} \frac{c^2}{6} \left( 1 - \left( \frac{1 - (x/c)^2}{3} \right) \right) &amp; \text{if }</td>
<td>x</td>
<td>\leq c \ \frac{c^2}{6} &amp; \text{if }</td>
</tr>
<tr>
<td>Andrews</td>
<td>( \begin{cases} k^2 (1 - \cos(x/k)) &amp; \text{if }</td>
<td>x</td>
<td>\leq k\pi \ 2k^2 &amp; \text{if }</td>
</tr>
</tbody>
</table>

### Table 2: Default tuning constants (\( k \) or \( c \)) for M-estimation \( \rho, \psi \) and weight functions.

<table>
<thead>
<tr>
<th>Method</th>
<th>Tuning Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huber</td>
<td>1.345</td>
</tr>
<tr>
<td>fair</td>
<td>1.3998</td>
</tr>
<tr>
<td>Cauchy</td>
<td>2.3849</td>
</tr>
<tr>
<td>Welsch</td>
<td>2.9846</td>
</tr>
<tr>
<td>Tukey</td>
<td>4.6851</td>
</tr>
<tr>
<td>Andrews</td>
<td>1.339</td>
</tr>
</tbody>
</table>
\[ V = X^T \Psi' X \]  

(11)

and \( \Psi' \) is a diagonal matrix of \( \psi' \) values. When using `fitPLM` these are selected using `se.type=1`, `se.type=2`, or `se.type=3` respectively. Treating the regression as a weighted least squares problem would give

\[
\sum \frac{w(r_i)r_i^2}{n-p} \left( X^T W X \right)^{-1}
\]

(12)

as the estimator for variance covariance matrix, where \( W \) is a diagonal matrix of weight values. This option is selected by using `se.type=4`.

References


