msmsTests package
Blocks design to compensate batch effects

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1 Introduction
This vignette exemplifies the use of the packages msmsEDA and msmsTests in discover-
ing and correcting batch effects in label-free LC-MS/MS data based on spectral counts. Label-free experiments are specially sensitive to these effects as each condition has to be measured separately and may be influenced by uncontrolled factors in a different extend.

2 Dataset
This dataset [1] is the result of spiking experiments, showing real LC-MS/MS data. Sam-
ples of 500 micrograms of a standard yeast lysate are spiked with 200 and 600fm of a complex mix of 48 equimolar human proteins (UPS1, Sigma-Aldrich). The dataset comes with the package msmsEDA [2], and was used to evidence batch effects by exploratory
data analysis tools [3]. The dataset consists in an instance of the \textit{MSnSet} class, defined in the MSnbase package [4], a S4 class [5] [6]. This \textit{MSnSet} object contains a spectral counts (SpC) matrix in the \textit{assayData} slot, and factors treatment and batch in the \textit{phenoData} slot. (See also the expressionSet vignette by vignette("ExpressionSetIntroduction","package="Biobase") [7])

```r
> library(msmsTests)
> data(msms.dataset)
> msms.dataset

\textbf{MSnSet} (storageMode: lockedEnvironment)
\textbf{assayData}: 697 features, 14 samples
\hspace{1cm} \textbf{element names: exprs}
\textbf{protocolData}: none
\textbf{phenoData}
\hspace{1cm} \textbf{sampleNames}: U2.2502.1 U2.2502.2 ... U6.0302.3 (14 total)
\hspace{1cm} \textbf{varLabels}: treat batch
\hspace{1cm} \textbf{varMetadata}: labelDescription
\textbf{featureData}: none
\textbf{experimentData}: use \texttt{\'experimentData(object)\'}

\textbf{Annotation}:
\hspace{1cm} \texttt{-- Processing information --}
\hspace{1cm} MSnbase version: 1.8.0

> msms.counts <- exprs(msms.dataset)
> dim(msms.counts)
\[1\] 697 14

> table(pData(msms.dataset)$treat,pData(msms.dataset)$batch)

\begin{tabular}{llll}
   & 0302 & 2502 \\
U200 & 3 & 4 \\
U600 & 3 & 4 \\
\end{tabular}

Although the mix is equimolar the signal strength of each protein is markedly different, allowing to cover a wide range of SpC values, what makes it specially worth in this sort of experiments:

```r
> idx <- grep("HUMAN",featureNames(msms.dataset))
> mSpC <- t( apply(msms.counts[idx,],1,function(x)
     tapply(x,pData(msms.dataset)$treat,mean)) )

> apply(mSpC,2,summary)

\begin{tabular}{llll}
   & U200 & U600 \\
Min.  & 0.2857143 & 1.142857 \\
1st Qu. & 2.4642857 & 6.071429 \\
Median & 4.5714286 & 12.214286 \\
Mean & 5.9378882 & 15.161491 \\
3rd Qu. & 6.9642857 & 20.500000 \\
Max. & 18.8571429 & 47.000000 \\
\end{tabular}

2
3 Batch effects

Real life LC-MS/MS experiments use to be complicated enough to be able to get all required technical or biological replicates in a single batch run. Commonly a dataset collects results from multiple batches. The batches may be influenced by factors which escape our control capacity, and typically these datasets show the so known ’batch effects’ when the runs where obtained in different dates. The confounding caused by these effects is easily evidenced by multidimensional tools like Principal Components Analysis (PCA) or Hierarchical Clustering (HC), when the samples cluster by batches instead of by treatment [3] [8].

> snms <- substr(as.character(pData(msms.dataset)$treat),1,2)
> snms <- paste(snms,as.integer(pData(msms.dataset)$batch),sep=".")
> smpl.pca <- counts.pca(msms.dataset,snms=snms)$pca

![Figure 1: Principal Components Analysis](image)

4 Results on a single batch

The next code shows the results obtained from the data of a single batch with four replicates in each condition. The statistic test used for differential expression is the quasi-likelihood GLM [9] [10], and the p-values are adjusted with FDR control by the Benjamini-Hochberg [11] method. The quality of the results is given by a truth table.

> ### Subset and pre-process dataset
> fl <- pData(msms.dataset)$batch="2502"
> e <- msms.dataset[,fl]
> e <- pp.msms.data(e)
> ### Null and alternative model
### Normalizing condition

```r
counts <- exprs(e)
div <- apply(counts,2,sum)
```

### Quasi-likelihood GLM

```r
ql.res <- msms.glm.qlll(e,alt.f,null.f,div=div)
```

### Adjust p-values with FDR control.

```r
adjp <- p.adjust(ql.res$p.value,method="BH")
```

### Truth table

```r
nh <- length(grep("HUMAN",featureNames(e)))
ny <- length(grep("HUMAN",featureNames(e),invert=TRUE))
tp <- length(grep("HUMAN",rownames(ql.res)[adjp<=0.05]))
fp <- sum(adjp<=0.05)-tp
(tt.ql1 <- data.frame(TP=tp,FP=fp,TN=ny-fp,FN=nh-tp))
```

<table>
<thead>
<tr>
<th>TP</th>
<th>FP</th>
<th>TN</th>
<th>FN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>4</td>
<td>571</td>
</tr>
</tbody>
</table>

These results may be polished by a post-test filter, so that only relevant features are accepted as differentially expressed, and the false positives are further restricted [1].

```r
### Post-test filter

ql.tbl <- test.results(ql.res,e,pData(e)$treat,"U600","U200",div,
                       alpha=0.05,minSpC=2,minLFC=1,method="BH")$tres
ql.nms <- rownames(ql.tbl)[ql.tbl$DEP]
```

### Truth table

```r
ridx <- grep("HUMAN",ql.nms)
tp <- length(ridx)
fp <- length(ql.nms)-length(ridx)
(tt.ql11 <- data.frame(TP=tp,FP=fp,TN=ny-fp,FN=nh-tp))
```

<table>
<thead>
<tr>
<th>TP</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>0</td>
<td>575</td>
</tr>
</tbody>
</table>

5 Results on the global dataset

With a higher number of replicates the tests become more sensitive, and a higher number of differentially expressed features may be identified. The next code explores the full dataset, composed of two batches and seven replicates of each condition. Again the quality of the results is given by a truth table.

```r
### Pre-process dataset

gble <- pp.msms.data(msms.dataset)
```

### Null and alternative model

```r
null.f <- "y~1"
```
### Normalizing condition

```r
div <- apply(exprs(gble),2,sum)
```

### Quasi-likelihood GLM

```r
ql.res <- msms.glm.qllll(gble,alt.f,null.f,div=div)
```

### Adjust p-values with FDR control.

```r
adjp <- p.adjust(ql.res$p.value,method="BH")
```

### Truth table

```r
nh <- length(grep("HUMAN",featureNames(gble)))
ny <- length(grep("HUMAN",featureNames(gble),invert=TRUE))
tp <- length(grep("HUMAN",rownames(ql.res)[adjp<=0.05]))
fp <- sum(adjp<=0.05)-tp
(tt.ql2 <- data.frame(TP=tp,FP=fp,TN=ny-fp,FN=nh-tp))
```

<table>
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<th>TN</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>1</td>
<td>628</td>
</tr>
</tbody>
</table>

Applying a post-test filter, as before, the results become:

```r
### Post-test filter

```r
ql.tbl <- test.results(ql.res,gble,pData(gble)$treat,"U600","U200",div,
                       alpha=0.05,minSpC=2,minLFC=1,method="BH")$tres
```

```r
ql.nms <- rownames(ql.tbl)[ql.tbl$DEP]
```

### Truth table

```r
ridx <- grep("HUMAN",ql.nms)
tp <- length(ridx)
fp <- length(ql.nms)-length(ridx)
(tt.ql22 <- data.frame(TP=tp,FP=fp,TN=ny-fp,FN=nh-tp))
```

<table>
<thead>
<tr>
<th>TP</th>
<th>FP</th>
<th>TN</th>
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<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>0</td>
<td>629</td>
</tr>
</tbody>
</table>

6 Results on the global dataset with a blocking factor

When the batches are balanced in the treatment conditions the presence of confounding factors translates into bigger variance and lower sensitivity. We may account for this extra variability by introducing the batches into the model, as a blocking factor. The next code explores the corresponding results.

```r
### Null and alternative model

```r
null.f <- "y~batch"
```

```r
alt.f <- "y~treat+batch"
```

### Quasi-likelihood GLM

```r
ql.res <- msms.glm.qllll(gble,alt.f,null.f,div=div)
```

### Adjust p-values with FDR control.

```r
adjp <- p.adjust(ql.res$p.value,method="BH")
```
> ### Truth table
> nh <- length(grep("HUMAN", featureNames(gble)))
> ny <- length(grep("HUMAN", featureNames(gble), invert=TRUE))
> tp <- length(grep("HUMAN", rownames(ql.res)[adjp<=0.05]))
> fp <- sum(adjp<=0.05)-tp
> (tt.ql3 <- data.frame(TP=tp, FP=fp, TN=ny-fp, FN=nh-tp))

<table>
<thead>
<tr>
<th>TP</th>
<th>FP</th>
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<th>FN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>18</td>
<td>611</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The correction improved the number of true positives, but significantly increased the number of false positives. This may be polished by the post-test filter to remove the non relevant features:

> ### Post-test filter
> ql.tbl <- test.results(ql.res, gble, pData(gble)$treat, "U600", "U200", div, alpha=0.05, minSpC=2, minLFC=1, method="BH")$tres
> ql.nms <- rownames(ql.tbl)[ql.tbl$DEP]
> ### Truth table
> ridx <- grep("HUMAN", ql.nms)
> tp <- length(ridx)
> fp <- length(ql.nms)-length(ridx)
> (tt.ql33 <- data.frame(TP=tp, FP=fp, TN=ny-fp, FN=nh-tp))

<table>
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<th>TP</th>
<th>FP</th>
<th>TN</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>0</td>
<td>629</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7 Comparison of results

The following table collects the results obtained so far, where we see how increasing the number of replicates we improve the sensitivity, how the use of a post-test filter helps in restricting the number of false positives, and how blocking helps to remove the extra variability introduced by batch effects.

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>FP</th>
<th>TN</th>
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</tr>
</thead>
<tbody>
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<td>subset</td>
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<td>571</td>
<td>22</td>
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<td>subset filtered</td>
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<td>blocking filtered</td>
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<td>0</td>
<td>629</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 1: Truth tables
Figure 2: Comparison of results
References


