

Affy array outlier detection via dimension reduction

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1 Introduction

Clinical trials groups now routinely produce hundreds of microarrays to generate measures of clinical conditions and treatment responses at the level of mRNA abundance. Objective, quantitative measures of array quality are important to support these projects. Numerous packages in Bioconductor address quality assessment procedures. *ArrayQualityMetrics* is a particularly attractive set of tools. We provide *arrayMvout* as a module that performs parametric outlier detection after data reduction to support formal decisionmaking about array acceptability. Ultimately the measures and procedures provided by *arrayMvout* may be useful as components of other packages for quality assessment. Another closely related package is *mdqc*, which employs a variety of robustifications of Mahalanobis distance to help identify outlying arrays.

Suppose there are N affymetrix arrays to which N independent samples have been hybridized. The *arrayMvout* package computes Q quality measures which constitute

array-specific features. These features are then analyzed in two steps. First, principal components analysis is applied to the $N \times Q$ feature matrix. Second, parametric multivariate outlier detection with calibration for multiple testing is applied to a subset of the resulting principal components. Arrays identified as outliers by this procedure are then subject to additional inspection and/or exclusion as warranted.

In this vignette we illustrate application of the procedure for a ‘negative control’ (raw MAQC data) and several constructed quality defect situations.

2 Illustration with MAQC data

We have serialized sufficient information on the MAQC subset to allow a simple demonstration of a negative control set. The MAQC data should be free of outliers.

We will manually search for outliers in this data resource. We compute principal components and take the first three.

```
> library(arrayMvout)
> data(maqcQA)
> mm = ArrayOutliers(maqcQA[, 3:11], alpha=.01)
> mm
```

ArrayOutliers result.

The call was:

```
.local(data = data, alpha = alpha, alphaSeq = alphaSeq)
```

No outliers. First row of QC features

	avgBG	SF	Present	HSAC07	GAPDH	NUSE	RLE	RLE_IQR
1	60.05505	1.17657	52.4225	1.245477	1.015217	1.057659	0.04185417	0.5516266
	RNAslope							
1	3.141527							

There are no outliers found at a false labeling rate of 0.01.

3 Illustration with arrays from a clinical trial network

Another data resource with some problematic arrays is also included.

```
> data(itnQA)
> ii = ArrayOutliers(itnQA, alpha=.01)
> ii
```

ArrayOutliers result.

The call was:

```
.local(data = data, alpha = alpha, alphaSeq = alphaSeq)
```

There were 507 samples with 18 outliers detected.

Coordinate-wise means of inlying arrays:

avgBG	SF	Present	HSAC07	GAPDH	NUSE
4.498102e+01	1.731430e+00	4.162066e+01	2.187432e+00	1.770430e+00	1.000867e+00
RLE	RLE_IQR	RNAslope			
6.050559e-05	2.976345e-01	3.427889e+00			

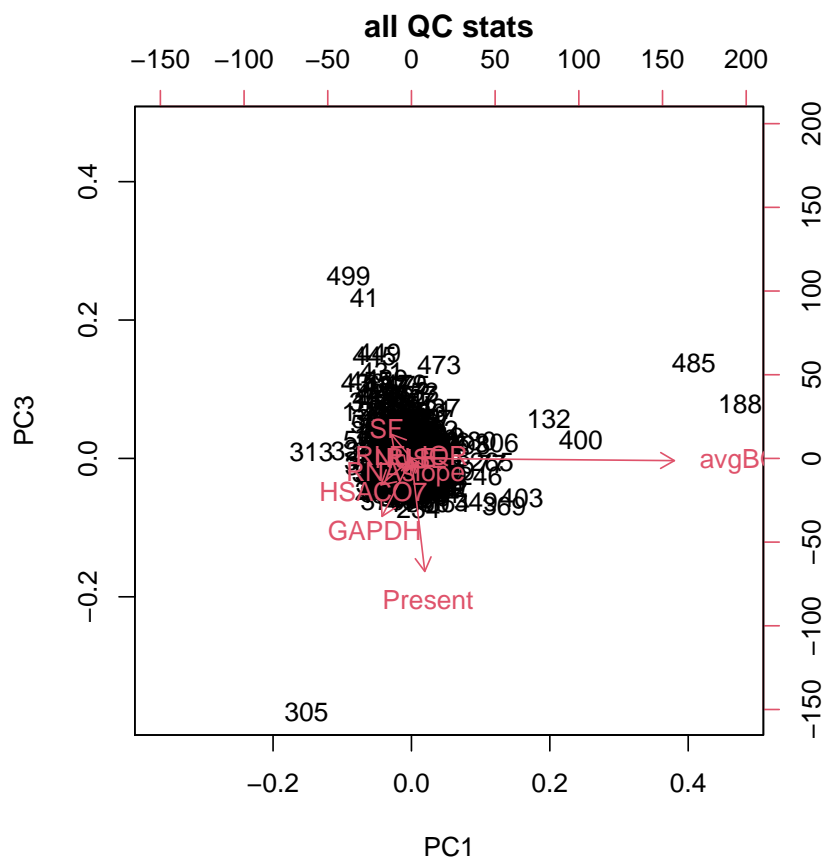
Features of outlying arrays:

	avgBG	SF	Present	HSAC07	GAPDH	NUSE	RLE
403	77.07796	0.4374264	47.55007	1.433827	1.307327	0.9991413	0.005285121
16	41.00919	1.9288207	39.60311	12.647909	4.707294	1.0097374	0.021545990
449	37.26800	6.2588701	24.39140	4.131615	2.047267	1.0284928	0.127501020
189	40.61447	1.9891220	39.39095	10.360385	6.718598	1.0103497	0.036462661
445	36.28049	9.1599338	25.08642	5.930873	2.189993	1.1064651	0.036035969
473	54.36467	3.6423721	26.03567	2.497312	1.893548	1.0438432	0.108838227
235	40.36647	2.3888050	39.61957	8.852826	7.201938	1.0302444	0.051526272
323	31.73768	5.6643255	32.43347	16.760480	11.955661	1.0507761	0.052612806
268	50.32263	2.6636200	34.85871	23.990430	7.101770	1.0542473	0.092065904
274	39.08553	2.9476038	36.66575	22.884391	12.036149	1.0457600	0.087581891
132	85.82265	1.8183446	34.61363	1.432298	1.510193	1.0822086	0.009663181
499	30.14740	29.3554869	17.02423	2.629898	1.718535	1.0874808	0.132794452
41	33.71813	15.6434771	17.80887	1.073282	2.643669	1.1669600	0.321298991
400	95.45017	1.2115402	36.64563	3.508757	2.479803	1.1128690	-0.000687765
485	129.10624	1.5643922	23.87197	3.057225	1.810761	1.1098076	0.073777325
188	142.44325	1.4429218	30.23137	2.907469	2.101054	1.1475918	-0.018917415
313	25.37851	16.8069277	25.72474	56.917203	11.840316	1.1360326	0.073802881
305	38.11554	19.0772560	11.60677	67.021339	120.011952	1.1987268	0.349949413
	RLE_IQR	RNAslope					
403	0.2197193	3.66807105					
16	0.3299879	5.42259351					
449	0.5672631	1.89557125					
189	0.3305528	5.80774028					
445	0.5465581	2.54468591					
473	0.6448400	1.32173147					
235	0.3657481	6.54325480					
323	0.4407913	5.88500524					
268	0.5755214	5.74973052					
274	0.4367455	7.09471717					
132	0.6664186	1.19952480					
499	0.6250615	1.87786720					
41	1.0754575	1.11521819					
400	0.6759216	2.46627267					
485	0.9486388	-0.04085089					

```
188 0.8908112 1.76393357
313 0.6503561 6.13572050
305 1.3208768 6.96678147
```

We have a simple visualization.

```
> plot(ii, choices=c(1,3))
```



4 Manual work with the MAQC subset

The remaining text of this vignette is computed statically. The source code with `eval=FALSE` is given as an appendix.

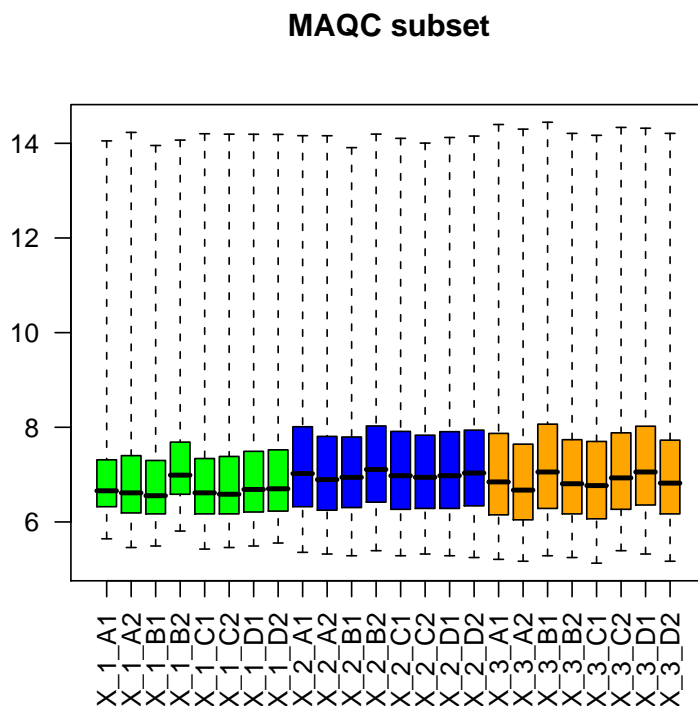
We consider an `AffyBatch` supplied with the Bioconductor `MAQCsubset` package. Marginal boxplots of raw intensity data are provided in the next figure. Sample labels are decoded `AFX - [lab] - [type] [replicate] .CEL` where `[lab] ∈ {1, 2, 3}`, `[type]` denotes mixture type (A = 100% USRNA, B = 100% Ambion brain, C = 75% USRNA, 25% brain, D = 25% USRNA, 75% brain), and `[replicate] ∈ {1, 2}`.

```

> library(arrayMvout)
> library(MAQCsubset)
> if (!exists("afxsub")) data(afxsub)
> sn = sampleNames(afxsub)
> if (nchar(sn)[1] > 6) {
+   sn = substr(sn, 3, 8)
+   sampleNames(afxsub) = sn
+ }

> opar = par(no.readonly = TRUE)
> par(mar = c(10, 5, 5, 5), las = 2)
> boxplot(afxsub, main = "MAQC subset", col = rep(c("green", "blue",
+   "orange"), c(8, 8, 8)))
> par(opar)

```



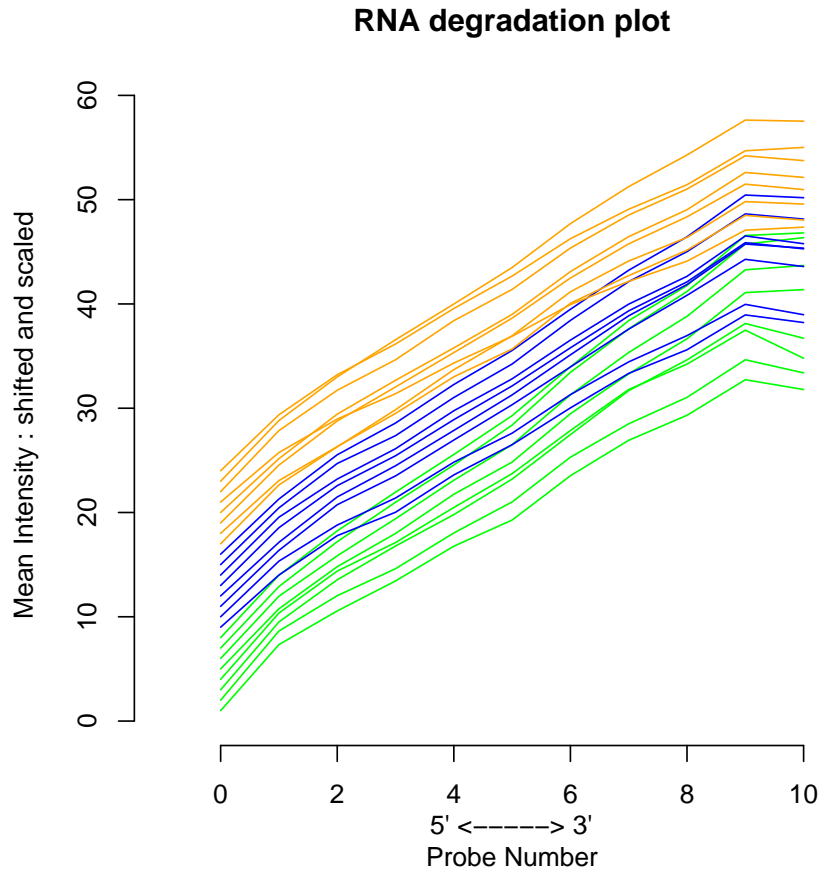
4.1 QA diagnostics

Of interest are measures of RNA degradation:

```

> library(arrayMvout)
> data(afxsubDEG)
> plotAffyRNAdeg(afxsubDEG, col = rep(c("green", "blue", "orange"),
+   c(8, 8, 8)))

```



and the general 'simpleaffy' QC display:

```

> data(afxsubQC)
> plot(afxsubQC)

```



The affyPLM package fits probe-level robust regressions to obtain probe-set summaries.

```
> library(affyPLM)
> splm = fitPLM(afxsub)

> png(file = "doim.png")
> par(mar = c(7, 5, 5, 5), mfrow = c(2, 2), las = 2)
> NUSE(splm, ylim = c(0.85, 1.3))
> RLE(splm)
> image(splm, which = 2, type = "sign.resid")
> image(splm, which = 5, type = "sign.resid")
```

In the following graphic, we have the NUSE distributions (upper left), the RLE distributions (upper right), and second and fifth chips in signed residuals displays.



These chips seem to have adequate quality, although there is some indication that the first four are a bit different with respect to variability.

4.2 Outlier detection using diagnostics

Let's apply the diagnostic-dimension reduction-multivariate outlier procedure `ArrayOutliers`.

```
> AO = ArrayOutliers(afxsub, alpha = 0.05, qcOut = afxsubQC, plmOut = splm,
+   degOut = afxsubDEG)
> nrow(AO[["out1"]])

[1] 0
```

We see that there are no outliers declared. This seems a reasonable result for arrays that were hybridized in the context of a QC protocol. Let us apply the `mdqc` procedure. As input this takes any matrix of quality indicators. The third component of our `ArrayOutliers` result provides these as computed using `simpleaffy qc()`, `affy AffyRNAdeg`, and `affyPLM NUSE` and `RLE`. The QC measures for the first two chips are:


```
> AO[[3]][1:2, ]
```

	avgBG	SF	Present	HSAC07	GAPDH	NUSE	RLE
X_1_A1	60.05505	1.1765695	52.42250	1.245477	1.065898	1.064069	0.04163564
X_1_A2	52.42248	0.9459093	54.63009	1.273977	1.094208	1.040718	0.03253112
	RLE_IQR	RNAslope					
X_1_A1	0.5626532	3.141527					
X_1_A2	0.5459847	3.210157					

We now use the mdqc package with MVE robust covariance estimation.

```
> library(mdqc)
> mdq = mdqc(AO[[3]], robust = "MVE")
> mdq
```

```
Method used: nogroups          Number of groups: 1
Robust estimator: MVEMDs exceeding the square root of the  90 % percentile of the Chi-S
[1]  6 16 17 18 21 24
MDs exceeding the square root of the  95 % percentile of the Chi-Square distribution
[1]  6 16 17 18 21 24
MDs exceeding the square root of the  99 % percentile of the Chi-Square distribution
[1]  6 16 17 18 21 24
```

We see that a number of the arrays are determined to be outlying by this procedure according to several thresholds.

5 Intensity contamination in the spikein data

We begin with a simple demonstration of a contamination procedure that simulates severe blobby interference with hybridization.

The code below is unevaluated to speed execution. Set eval=TRUE on all chunks to see the actual process.

```
> require(mvoutData)
> data(s12c)

> image(s12c[, 1])
```

12_13_02_U133A_Mer_Latin_Square_Expt1_R1



For this AffyBatch instance, we have contaminated the first two arrays in this way. We now apply the ArrayOutliers procedure:

```
> aos12c = ArrayOutliers(s12c, alpha = 0.05)
```

```
> aos12c[[1]]
```

	samp	avgBG	SF	Present		
1	12_13_02_U133A_Mer_Latin_Square_Expt1_R1	7205.48413	5.494978	19.05381		
2	12_13_02_U133A_Mer_Latin_Square_Expt2_R1	7205.12544	5.801398	17.62332		
8	12_13_02_U133A_Mer_Latin_Square_Expt8_R1	31.23121	1.181946	45.47982		
	HSAC07	GAPDH	NUSE	RLE	RLE_IQR	RNASlope
1	9.488671	8.6364494	1.578471	-0.514913919	1.33150311	-0.05195296
2	8.517500	9.7475845	1.579164	-0.514617696	1.37213796	-0.07638096
8	1.042255	0.8965249	1.000006	0.001987620	0.09164597	1.53050152

We find three arrays declared to be outlying. At the different candidate significance levels we have:

```

> aos12c[[4]]

[[1]]
[[1]]$inds
[1] 1 2

[[1]]$vals
      PC1      PC2      PC3
[1,] -6.345625  0.08184738  0.05419247
[2,] -6.485447 -0.07281758 -0.05421514

[[1]]$k
[1] 5

[[1]]$alpha
[1] 0.01


[[2]]
[[2]]$inds
[1] 8 1 2

[[2]]$vals
      PC1      PC2      PC3
[1,]  1.104062 -0.18796407 -0.008319271
[2,] -6.345625  0.08184738  0.054192469
[3,] -6.485447 -0.07281758 -0.054215145

[[2]]$k
[1] 5

[[2]]$alpha
[1] 0.05


[[3]]
[[3]]$inds
[1] 8 1 2

[[3]]$vals
      PC1      PC2      PC3
[1,]  1.104062 -0.18796407 -0.008319271
[2,] -6.345625  0.08184738  0.054192469

```

```
[3,] -6.485447 -0.07281758 -0.054215145
```

```
[[3]]$k  
[1] 5
```

```
[[3]]$alpha  
[1] 0.1
```

So at the 0.01 level we have identified only the contaminated arrays.

We apply `mdqc` in the same manner.

```
> mdqc(aos12c[[3]], robust = "MVE")
```

```
Method used: nogroups          Number of groups: 1  
Robust estimator: MVEMDs exceeding the square root of the 90 % percentile of the Chi-S  
[1] 2  
MDs exceeding the square root of the 95 % percentile of the Chi-Square distribution  
[1] 2  
MDs exceeding the square root of the 99 % percentile of the Chi-Square distribution  
[1] 2
```

We see that only one of the contaminated arrays is identified by this procedure. This may be an instance of masking.

6 Appendix: Sources and text for statically computed sections with `eval` set to `false`

Manual work with the MAQC subset

All the code following has had evaluation turned off because execution times are slow.

We consider an `AffyBatch` supplied with the Bioconductor `MAQCsubset` package. Marginal boxplots of raw intensity data are provided in the next figure. Sample labels are decoded `AFX - [lab] - [type] [replicate] .CEL` where `[lab]` $\in (1, 2, 3)$, `[type]` denotes mixture type (A = 100% USRNA, B = 100% Ambion brain, C = 75% USRNA, 25% brain, D = 25% USRNA, 75% brain), and `[replicate]` $\in (1, 2)$.

```
> library(arrayMvout)  
> library(MAQCsubset)  
> if (!exists("afxsub")) data(afxsub)  
> sn = sampleNames(afxsub)  
> if (nchar(sn)[1] > 6) {  
+   sn = substr(sn, 3, 8)
```

```

+   sampleNames(afxsub) = sn
+ }

> opar = par(no.readonly=TRUE)
> par(mar=c(10,5,5,5), las=2)
> boxplot(afxsub, main="MAQC subset",
+   col=rep(c("green", "blue", "orange"), c(8,8,8)))
> par(opar)

```

QA diagnostics

Of interest are measures of RNA degradation:

```

> #afxsubDEG = AffyRNAdeg(afxsub)
> #save(afxsubDEG, file="afxsubDEG.rda")
> library(arrayMvout)
> data(afxsubDEG)
> plotAffyRNAdeg(afxsubDEG,
+   col=rep(c("green", "blue", "orange"), c(8,8,8)))

```

and the general ‘simpleaffy’ QC display:

```

> #afxsubQC = qc(afxsub)
> #save(afxsubQC, file="afxsubQC.rda")
> data(afxsubQC)
> plot(afxsubQC)

```

The affyPLM package fits probe-level robust regressions to obtain probe-set summaries.

```

> library(affyPLM)
> #if (file.exists("splm.rda")) load("splm.rda")
> #if (!exists("splm")) splm = fitPLM(afxsub)
> splm = fitPLM(afxsub)
> #save(splm, file="splm.rda")

> png(file="doim.png")
> par(mar=c(7,5,5,5),mfrow=c(2,2),las=2)
> NUSE(splm, ylim=c(.85,1.3))
> RLE(splm)
> image(splm, which=2, type="sign.resid")
> image(splm, which=5, type="sign.resid")

```

These chips seem to have adequate quality, although there is some indication that the first four are a bit different with respect to variability.

Outlier detection using diagnostics

Let's apply the diagnostic-dimension reduction-multivariate outlier procedure `ArrayOutliers`.

```
> AO = ArrayOutliers(afxsub, alpha=0.05, qcOut=afxsubQC,
+   plmOut=splm, degOut=afxsubDEG)
> nrow(AO[["out1"]])
```

We see that there are no outliers declared. This seems a reasonable result for arrays that were hybridized in the context of a QC protocol. Let us apply the `mdqc` procedure. As input this takes any matrix of quality indicators. The third component of our `ArrayOutliers` result provides these as computed using `simpleaffy qc()`, `affy AffyRNAdeg`, and `affyPLM NUSE` and `RLE`. The QC measures for the first two chips are:

```
> AO[[3]][1:2, ]
```

We now use the `mdqc` package with MVE robust covariance estimation.

```
> library(mdqc)
> mdq = mdqc( AO[[3]], robust="MVE" )
> mdq
```

We see that a number of the arrays are determined to be outlying by this procedure according to several thresholds.

Intensity contamination in the spikein data

We begin with a simple demonstration of a contamination procedure that simulates severe blobby interference with hybridization.

The code below is unevaluated to speed execution. Set `eval=TRUE` on all chunks to see the actual process.

```
> require(mvoutData)
> data(s12c)

> image(s12c[,1])
```

For this `AffyBatch` instance, we have contaminated the first two arrays in this way. We now apply the `ArrayOutliers` procedure:

```
> aos12c = ArrayOutliers(s12c, alpha=0.05)

> aos12c[[1]]
```

We find three arrays declared to be outlying. At the different candidate significance levels we have:

```
> aos12c[[4]]
```

So at the 0.01 level we have identified only the contaminated arrays.

We apply mdqc in the same manner.

```
> mdqc(aos12c[[3]], robust="MVE")
```

We see that only one of the contaminated arrays is identified by this procedure. This may be an instance of masking.

```
> sessionInfo()
```

```
R version 4.0.0 alpha (2020-04-05 r78150)
Platform: x86_64-apple-darwin17.0 (64-bit)
Running under: macOS Mojave 10.14.6
```

```
Matrix products: default
```

```
BLAS: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
```

```
locale:
```

```
[1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
```

```
attached base packages:
```

```
[1] parallel tools stats graphics grDevices utils datasets
[8] methods base
```

```
other attached packages:
```

```
[1] arrayMvout_1.45.0 lumi_2.39.3 affy_1.65.1
[4] Biobase_2.47.3 BiocGenerics_0.33.3 parody_1.45.5
```

```
loaded via a namespace (and not attached):
```

```
[1] colorspace_1.4-1 ellipsis_0.3.0
[3] siggenes_1.61.0 mclust_5.4.5
[5] XVector_0.27.2 GenomicRanges_1.39.3
[7] base64_2.0 affyio_1.57.0
[9] bit64_0.9-7 AnnotationDbi_1.49.1
[11] fansi_0.4.1 xml2_1.3.0
[13] codetools_0.2-16 splines_4.0.0
[15] scrime_1.3.5 methylumi_2.33.0
[17] Rsamtools_2.3.7 annotate_1.65.1
```

[19] cluster_2.1.0	dbplyr_1.4.2
[21] HDF5Array_1.15.15	BiocManager_1.30.10
[23] readr_1.3.1	compiler_4.0.0
[25] httr_1.4.1	assertthat_0.2.1
[27] Matrix_1.2-18	limma_3.43.5
[29] cli_2.0.2	prettyunits_1.1.1
[31] glue_1.4.0	GenomeInfoDbData_1.2.2
[33] dplyr_0.8.5	rappdirs_0.3.1
[35] doRNG_1.8.2	Rcpp_1.0.4.6
[37] bumphunter_1.29.0	vctrs_0.2.4
[39] Biostrings_2.55.7	multtest_2.43.1
[41] preprocessCore_1.49.2	nlme_3.1-145
[43] rtracklayer_1.47.0	iterators_1.0.12
[45] DelayedMatrixStats_1.9.1	stringr_1.4.0
[47] lifecycle_0.2.0	rngtools_1.5
[49] XML_3.99-0.3	beanplot_1.2
[51] nleqslv_3.3.2	zlibbioc_1.33.1
[53] MASS_7.3-51.5	simpleaffy_2.63.0
[55] hms_0.5.3	SummarizedExperiment_1.17.5
[57] minfi_1.33.1	rhdf5_2.31.10
[59] GEOquery_2.55.1	RColorBrewer_1.1-2
[61] curl_4.3	memoise_1.1.0
[63] biomaRt_2.43.5	gcrma_2.59.0
[65] reshape_0.8.8	stringi_1.4.6
[67] RSQLite_2.2.0	genefilter_1.69.0
[69] S4Vectors_0.25.15	foreach_1.5.0
[71] GenomicFeatures_1.39.7	BiocParallel_1.21.2
[73] GenomeInfoDb_1.23.16	rlang_0.4.5
[75] pkgconfig_2.0.3	matrixStats_0.56.0
[77] bitops_1.0-6	nor1mix_1.3-0
[79] lattice_0.20-41	purrr_0.3.3
[81] Rhdf5lib_1.9.2	GenomicAlignments_1.23.2
[83] bit_1.1-15.2	tidyselect_1.0.0
[85] plyr_1.8.6	magrittr_1.5
[87] R6_2.4.1	IRanges_2.21.8
[89] DelayedArray_0.13.10	DBI_1.1.0
[91] pillar_1.4.3	mgcv_1.8-31
[93] survival_3.1-11	RCurl_1.98-1.1
[95] tibble_3.0.0	crayon_1.3.4
[97] KernSmooth_2.23-16	BiocFileCache_1.11.4
[99] progress_1.2.2	locfit_1.5-9.4
[101] grid_4.0.0	data.table_1.12.8

[103]	blob_1.2.1	digest_0.6.25
[105]	affyContam_1.45.0	xtable_1.8-4
[107]	tidyr_1.0.2	mdqc_1.49.0
[109]	illuminaio_0.29.0	openssl_1.4.1
[111]	stats4_4.0.0	askpass_1.1
[113]	quadprog_1.5-8	