

DASC user guide (PDF)

Haidong Yi†, Ayush T. Raman†, Han Zhang, Genevera Allen, Zhandong Liu

28 March 2017

Abstract

Batch effects are one of the major source of technical variations in high throughput studies such as omics profiling. It has been well established that batch effects can be caused by different experimental platforms, laboratory conditions, different sources of samples and personnel differences. These differences can confound the outcomes of interest and lead to spurious results. A critical input for batch correction algorithms are the knowledge of batch factors, which in many cases are unknown or inaccurate. Hence, the primary motivation of our paper is to detect hidden batch factors that can be used in standard techniques to accurately capture the relationship between expression and other modeled variables of interest. Here, we present *DASC*, a novel algorithm that is based on convex clustering and semi-NMF for the detection of unknown batch effects.

Package version: DASC 0.99.3

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1 Getting started

DASC is an R package distributed as part of the [Bioconductor](#) project. To install the package, start R and enter:

```
source("http://bioconductor.org/biocLite.R")
biocLite("DASC")
```

Please, make sure to that *NMF*, *cvxclustr* and *Biobase* are installed in your system before running *DASC*. Once *DASC* is installed, it can be loaded to the R environment by the following command.

2 Introduction

DASC is used for identifying batches and classifying samples into different batches in a high dimensional gene expression dataset. The batch information can be further used as a covariate in conjunction with other variables of interest among standard bioinformatics analysis like differential expression analysis.

2.1 Citation info

If you use *DASC* for your analysis, please cite it as here below. To cite package 'DASC' in publications use:

```
@Manual{,
  title = {DASC: Detecting hidden batch factors through data adaptive
    adjustment for biological effects.},
  author = {Haidong Yi, Ayush T. Raman, Han Zhang, Genevera I. Allen and
    Zhandong Liu},
  year = {2017},
  note = {R package version 0.1.0},
}
```

3 Quick Example

```
library(DASC)
data("esGolub")
samples <- c(20,21,28,30)
dat <- exprs(esGolub)[1:100,samples]
pdat <- pData(esGolub)[samples,]

## use nrun = 50 or more for better convergence of results
res <- convex_batch(edata = dat, pdata = pdat, factor = pdat$Cell,
  method = 'ama', type = 3, lambda = 1,
  rank = 2:3, nrun = 5, annotation="esGolub Dataset")
#consensusmap(res)
#plot(res)
```

4 Setting up the data

The first step in using DASC package is to properly format the data. For example, in case of gene expression data, it should be a matrix with features (genes, transcripts) in the rows and samples in the columns. DASC then requires the information for the variable of interest to model the gene expression data effectively. Variable of interest could be a genotype or treatment information.

4.1 Stanford Dataset

Below is an example of Stanford dataset (Chen et. al. PNAS, 2015; Gilad et. al. F1000 Research, 2015). Here, we use the filtered raw counts dataset that was published by Gilad et al. F1000 Research. 30% of genes with the lowest expression & mitochondrial genes were removed (Gilad et al.F1000 Research).

```
## libraries
set.seed(99999)
library(DESeq2)
library(ggplot2)
library(pcaExplorer)

## dataset
rawCounts <- stanfordData$rawCounts
metadata <- stanfordData$metadata

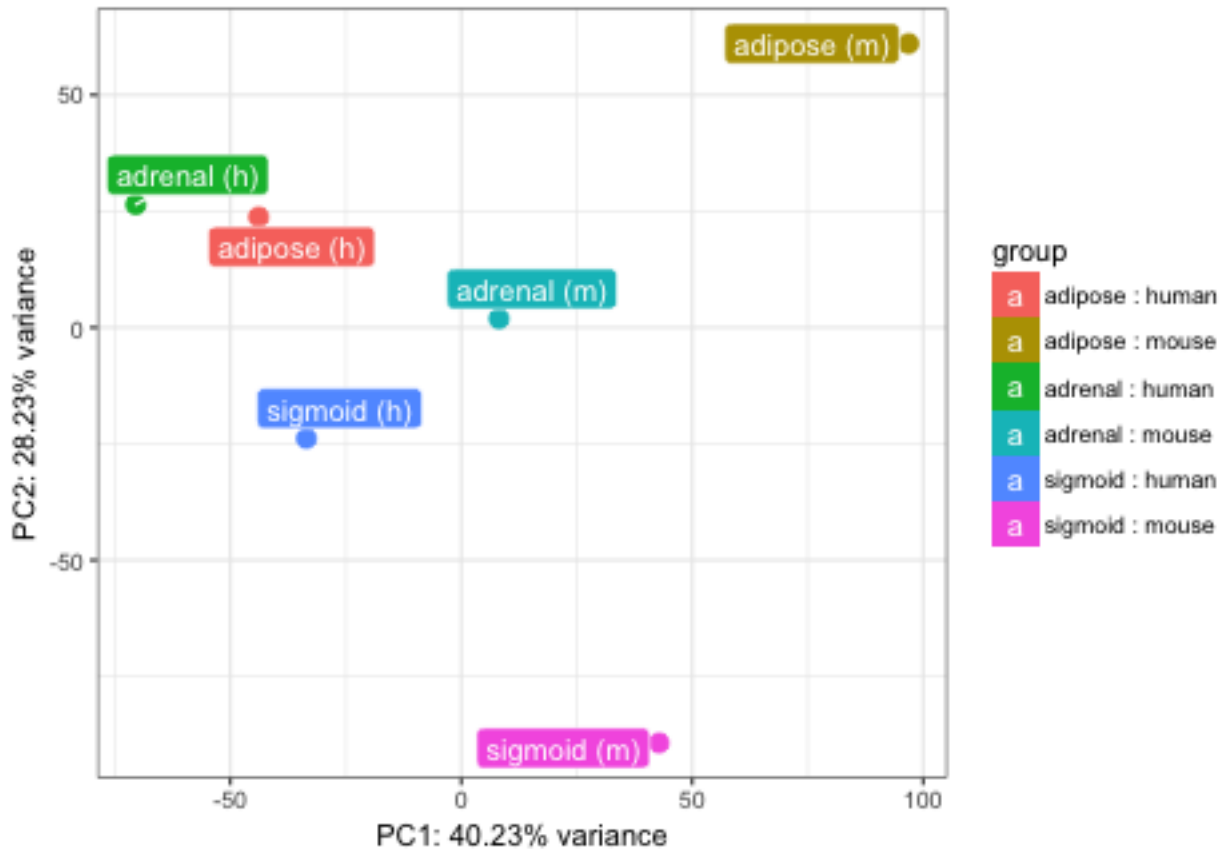
## Using a smaller dataset
idx <- which(metadata$tissue %in% c("adipose", "adrenal", "sigmoid"))
rawCounts <- rawCounts[,idx]
metadata <- metadata[idx,]
```

```
head(rawCounts)
##           adipose (h) adrenal (h) sigmoid (h) adipose (m) adrenal (m)
## STAG2           1430         4707         4392         3223         8235
## STAG1           835          2362          1687          2750          2732
## GOSR2           142           891           97           1599          1430
## C1orf43          1856          9591          2611           706           498
## ART5              1             4             0             0             0
## ART1              0             0             0             0             1
##           sigmoid (m)
## STAG2           10435
## STAG1           2833
## GOSR2            887
## C1orf43           753
## ART5              0
## ART1              0
head(metadata)
##           setname           seqBatch species tissue
## adipose (h) adipose (h) D87PMJN1:253:D2GUAACXX:8   human adipose
## adrenal (h) adrenal (h) D87PMJN1:253:D2GUAACXX:8   human adrenal
## sigmoid (h) sigmoid (h) D87PMJN1:253:D2GUAACXX:8   human sigmoid
## adipose (m) adipose (m) D4LHBFN1:276:C2HKJACXX:4   mouse adipose
## adrenal (m) adrenal (m) D4LHBFN1:276:C2HKJACXX:4   mouse adrenal
## sigmoid (m) sigmoid (m) D4LHBFN1:276:C2HKJACXX:4   mouse sigmoid
```

5 Batch detection using PCA Analysis

```
## Normalizing the dataset using DESeq2
dds <- DESeqDataSetFromMatrix(rawCounts, metadata, design = ~ species+tissue)
dds <- estimateSizeFactors(dds)
dat <- counts(dds, normalized = TRUE)
lognormalizedCounts <- log2(dat + 1)
```

```
## PCA plot using
rld.dds <- rlog(dds)
pcaplot(rld.dds, intgroup=c("tissue", "species"), ntop=1000, pcX=1, pcY=2)
```

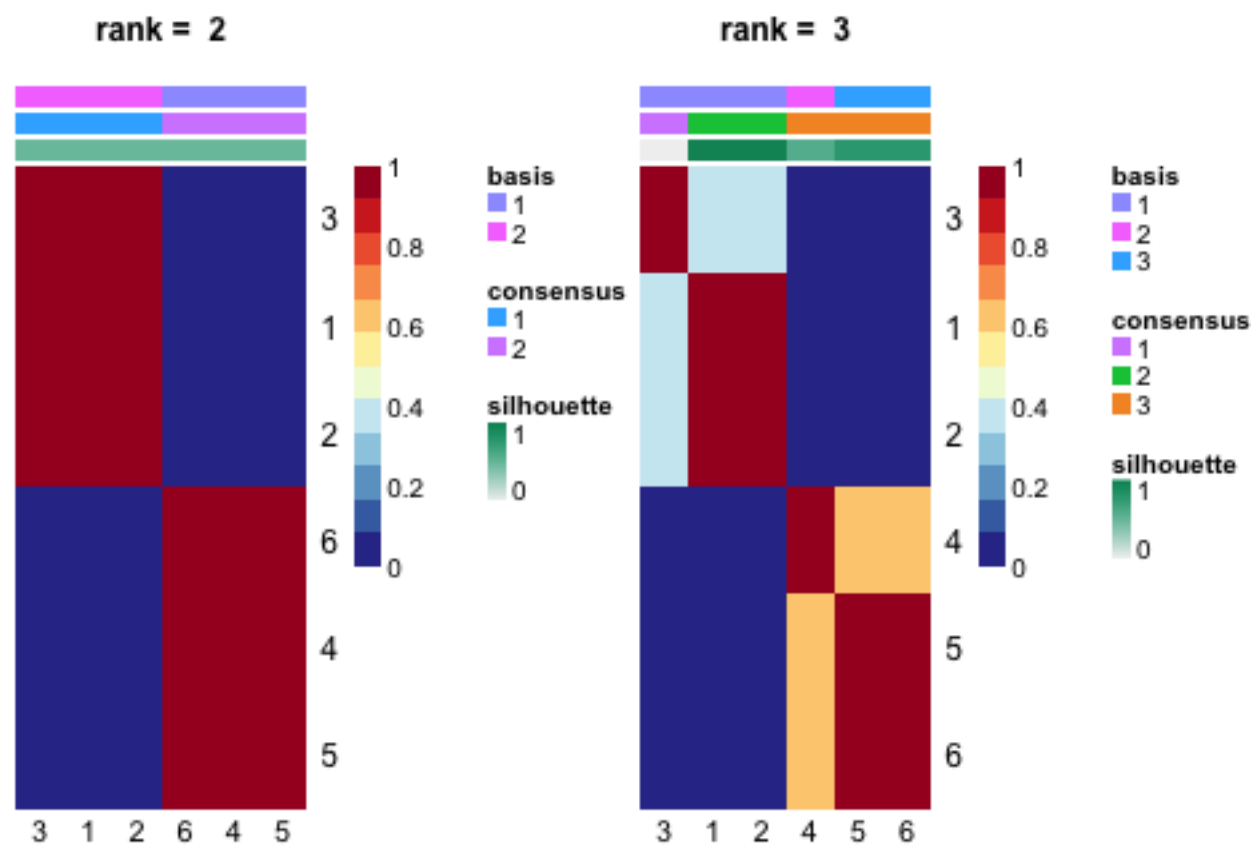


In the PCA plot, PC1 shows the differences between the species. PC2 shows the differences between the species i.e. samples clustering based on tissues.

6 Batch detection using DASC

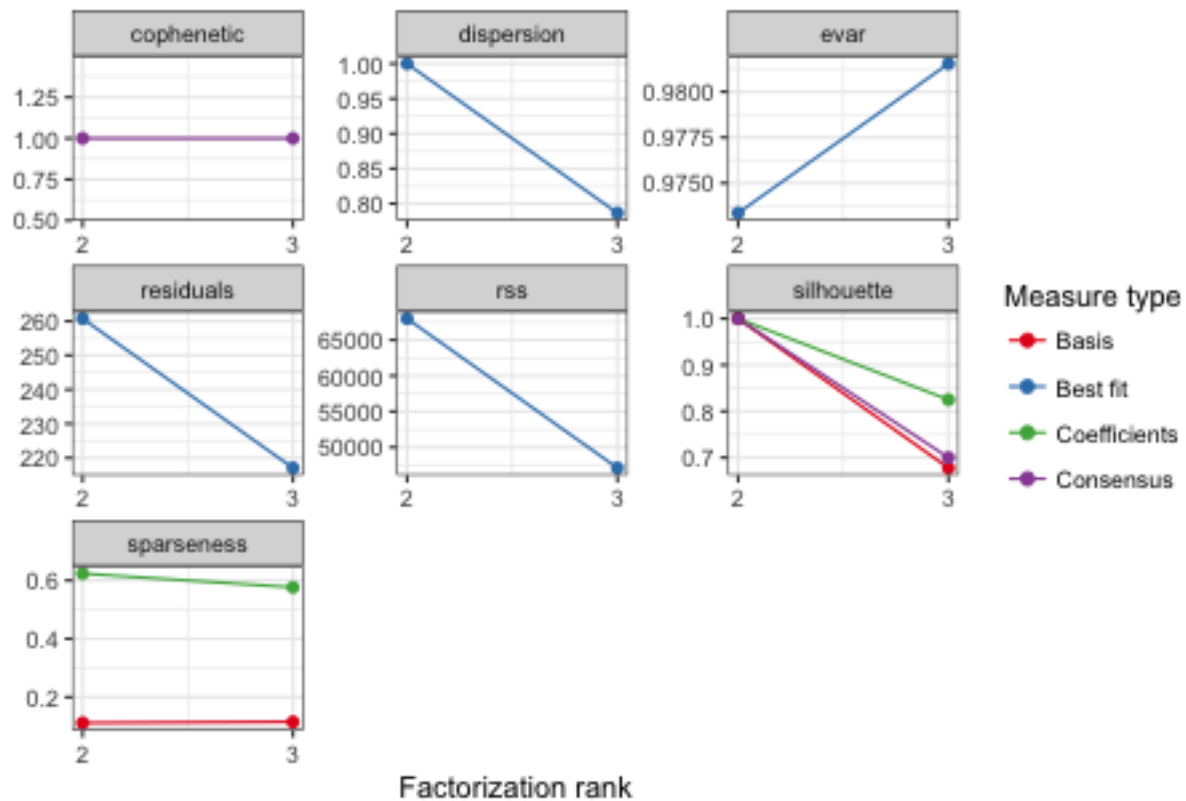
```
res <- convex_batch(edata = dat, pdata = metadata,
  factor = metadata$tissue, method = 'ama', type = 3,
  lambda = 1, rank = 2:3, nrun = 10,
  annotation = 'Stanford Dataset')
## Compute NMF rank= 2 ... + measures ... OK
## Compute NMF rank= 3 ... + measures ... OK

## Consensus plot
consensusmap(res)
```

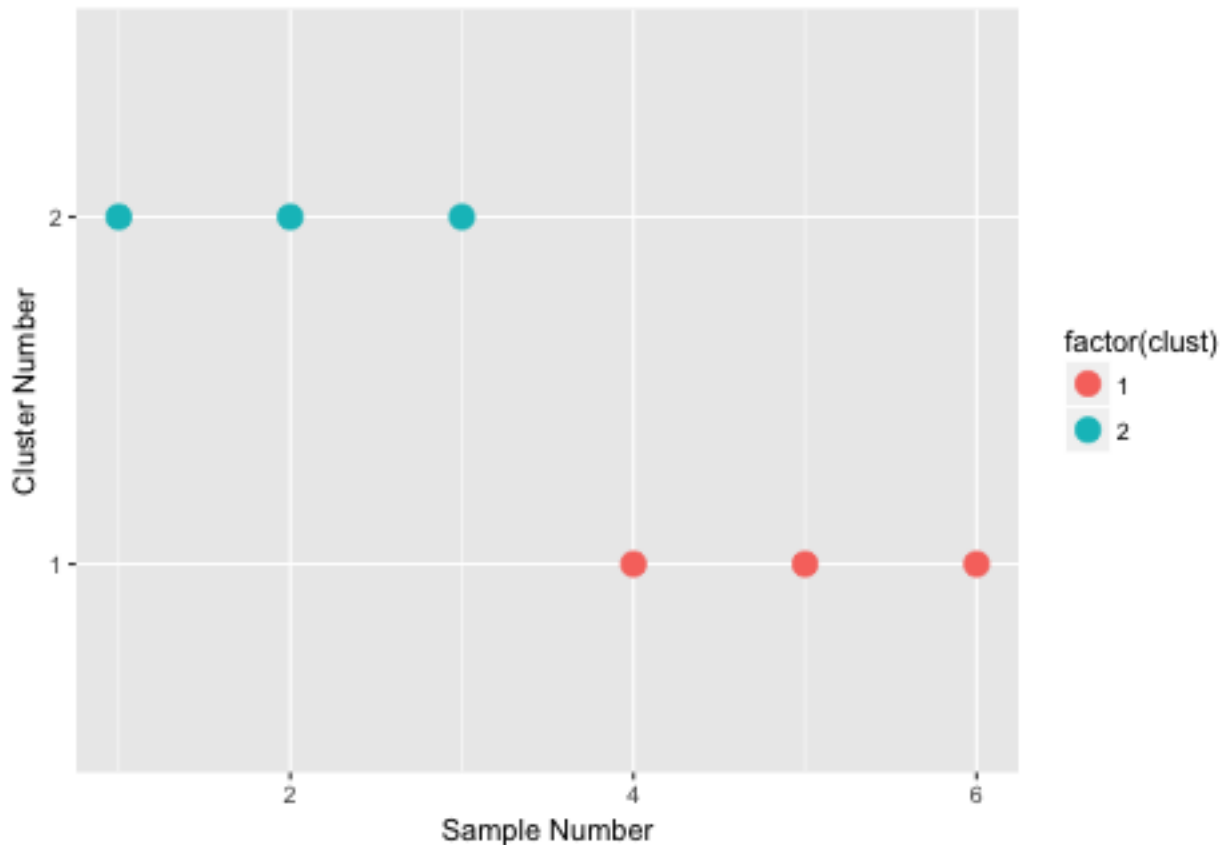


```
## Residual plot  
plot(res)
```

NMF rank survey



```
## Batches -- dataset has 6 batches
sample.clust <- data.frame(sample.name = colnames(lognormalizedCounts),
                           clust = as.vector(predict(res$fit$`2`)),
                           batch = metadata$seqBatch)
ggplot(data = sample.clust, aes(x=c(1:6), y=clust, color=factor(clust))) +
  geom_point(size = 4) + xlab("Sample Number") + ylab("Cluster Number")
```



Based on the above plots, we observe that the dataset has 2 batches. This can further be compared with the sequencing platform or `metadata$seqBatch`. The results suggest that differences in platform led to batch effects. Batch number can be used as another covariate, when differential expression analyses using `DESeq2`, `edgeR` or `limma` are performed.

7 More Examples

We are currently working on vignette and will be uploading more examples soon.

8 Session Info

```
sessionInfo()
## R version 3.3.2 (2016-10-31)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: macOS Sierra 10.12.3
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4      parallel  stats      graphics  grDevices  utils      datasets
## [8] methods    base
##
```

```

## other attached packages:
## [1] RColorBrewer_1.1-2          pcaExplorer_2.0.0
## [3] ggplot2_2.2.1              DESeq2_1.14.1
## [5] SummarizedExperiment_1.4.0 GenomicRanges_1.26.4
## [7] GenomeInfoDb_1.10.3        IRanges_2.8.2
## [9] S4Vectors_0.12.2           doParallel_1.0.10
## [11] iterators_1.0.8            foreach_1.4.3
## [13] DASC_0.99.3                 cvxclustr_1.1.1
## [15] igraph_1.0.1               Matrix_1.2-8
## [17] NMF_0.20.6                 cluster_2.0.6
## [19] rngtools_1.2.4             pkgmaker_0.22
## [21] registry_0.3               Biobase_2.34.0
## [23] BiocGenerics_0.20.0        BiocStyle_2.3.30
##
## loaded via a namespace (and not attached):
## [1] Category_2.40.0            bitops_1.0-6              matrixStats_0.51.0
## [4] threejs_0.2.2              rprojroot_1.2            tools_3.3.2
## [7] backports_1.0.5            R6_2.2.0                  DT_0.2
## [10] rpart_4.1-10               Hmisc_4.0-2              DBI_0.6
## [13] lazyeval_0.2.0             colorspace_1.3-2         nnet_7.3-12
## [16] gridExtra_2.2.1            compiler_3.3.2           graph_1.52.0
## [19] htmlTable_1.9              SparseM_1.76              labeling_0.3
## [22] d3heatmap_0.6.1.1          topGO_2.26.0             scales_0.4.1
## [25] checkmate_1.8.2            genefilter_1.56.0        RBGL_1.50.0
## [28] stringr_1.2.0              digest_0.6.12            shinyBS_0.61
## [31] foreign_0.8-67             rmarkdown_1.4            AnnotationForge_1.16.1
## [34] XVector_0.14.1             base64enc_0.1-3          htmltools_0.3.5
## [37] limma_3.30.13              htmlwidgets_0.8          RSQLite_1.1-2
## [40] shiny_1.0.0                GOstats_2.40.0           jsonlite_1.3
## [43] BiocParallel_1.8.1         acepack_1.4.1            RCurl_1.95-4.8
## [46] magrittr_1.5               GO.db_3.4.0              Formula_1.2-1
## [49] Rcpp_0.12.10              munsell_0.4.3            stringi_1.1.3
## [52] yaml_2.1.14               zlibbioc_1.20.0          plyr_1.8.4
## [55] grid_3.3.2                 ggrepel_0.6.5            shinydashboard_0.5.3
## [58] lattice_0.20-35            splines_3.3.2            annotate_1.52.1
## [61] locfit_1.5-9.1            knitr_1.15.1             geneplotter_1.52.0
## [64] reshape2_1.4.2            codetools_0.2-15         biomaRt_2.30.0
## [67] XML_3.98-1.5              evaluate_0.10            latticeExtra_0.6-28
## [70] data.table_1.10.4          png_0.1-7                httpuv_1.3.3
## [73] tidyr_0.6.1               gtable_0.2.0             assertthat_0.1
## [76] gridBase_0.4-7            mime_0.5                 xtable_1.8-2
## [79] survival_2.41-2           tibble_1.2-13            pheatmap_1.0.8
## [82] AnnotationDbi_1.36.2       memoise_1.0.0            GSEABase_1.36.0
## [85] shinyAce_0.2.1

```