

# Grade of Membership Model and Visualization for RNA-seq data using *CountClust*

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## Abstract

Grade of membership or GoM models (also known as admixture models or Latent Dirichlet Allocation”) are a generalization of cluster models that allow each sample to have membership in multiple clusters. It is widely used to model ancestry of individuals in population genetics based on SNP/ microsatellite data and also in natural language processing for modeling documents [?, ?].

This *R* package implements tools to visualize the clusters obtained from fitting topic models using a Structure plot [?] and extract the top features/genes that distinguish the clusters. In presence of known technical or batch effects, the package also allows for correction of these confounding effects.

**CountClust version:** 0.1.0 <sup>1</sup>

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<sup>1</sup>This document used the vignette from *Bioconductor* package *DESeq2*, *cellTree* as *knitr* template

## Contents

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### 1 Introduction

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In the context of RNA-seq expression (bulk or singlecell seq) data, the grade of membership model allows each sample to have some proportion of its RNA-seq reads coming from each cluster. For typical bulk RNA-seq experiments this assumption can be argued as follows: each sample is a mixture of different cell types, and so clusters could represent cell types (which are determined by the expression patterns of the genes), and the membership of a sample in each cluster could represent the proportions of each cell type present in that sample.

Many software packages available for document clustering are applicable to modeling RNA-seq data. Here, we use the R package `maptpx` [?] to fit these models, and we add functionality for visualizing the results and annotating clusters by their most distinctive genes to help biological interpretation. We also provide additional functionality to correct for batch effects and also compare the outputs from two different grade of membership model fits to the same set of samples but different in terms of feature description or model assumptions.

### 2 CountClust Installation

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*CountClust* requires the following CRAN-R packages: [maptpx](#), [slam](#), [ggplot2](#), [cowplot](#), [parallel](#) along with the *Bioconductor* package: [limma](#).

Installing *CountClust* from *Bioconductor* will install all these dependencies:

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

For installing the working version of this package and loading the data required for this vignette, we use CRAN-R package [devtools](#).

```
library(devtools)
install_github('kkdey/CountClust')
```

Then load the package with:

```
library(CountClust)
```

### 3 Data Preparation

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We load the data as summarized experiments for the GTEx (Genotype Tissue Expression) V6 Project Brain tissue samples [?], the Jaitin *et al* 2014 single cell data [?] and the Deng *et al* 2014 single cell

data across development stages of the mouse embryo [?].

### 3.1 Jaitin et al 2014

We install the Jaitin *et al* single cell data as a summarized experiment using *devtools*.

```
library(devtools)
install_github('https://github.com/jhsiao999/singleCellRNASeqMouseJaitinSpleen.git')

install_github('kkdey/singleCellRNASeqMouseDeng2014')
install_github('kkdey/GTExV6Brain')
```

We load the data.

```
library(singleCellRNASeqMouseJaitinSpleen)
jaitin.counts <- exprs(MouseJaitinSpleen)
jaitin.meta_data <- pData(MouseJaitinSpleen)
jaitin.gene_names <- rownames(jaitin.counts)
```

Extracting the non-ERCC genes satisfying some quality measures.

```
ENSG_genes_index <- grep("ERCC", jaitin.gene_names, invert = TRUE)
jaitin.counts_ensg <- jaitin.counts[ENSG_genes_index, ]
filter_genes <- c("M34473", "abParts", "M13680", "Tmsb4x",
                  "S100a4", "B2m", "Atpase6", "Rpl23", "Rps18",
                  "Rpl13", "Rps19", "H2-Ab1", "Rplp1", "Rpl4",
                  "Rps26", "EF437368")
fcounts <- jaitin.counts_ensg[ -match(filter_genes, rownames(jaitin.counts_ensg)), ]
sample_counts <- colSums(fcounts)

filter_sample_index <- which(jaitin.meta_data$number_of_cells == 1 &
                             jaitin.meta_data$group_name == "CD11c+" &
                             sample_counts > 600)
```

We filter the metadata likewise

```
jaitin.meta_data_filtered <- jaitin.meta_data[filter_sample_index, ]
```

### 3.2 Deng et al 2014

```
library(singleCellRNASeqMouseDeng2014)
deng.counts <- exprs(Deng2014MouseESC)
deng.meta_data <- pData(Deng2014MouseESC)
deng.gene_names <- rownames(deng.counts)
```

```
library(GTExV6Brain)
gtex.counts <- exprs(GTExV6Brain)
gtex.meta_data <- pData(GTExV6Brain)
gtex.gene_names <- rownames(gtex.counts)
```

## 4 Fitting GoM Model

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We use a wrapper function of the *topics()* function in the *maptpx* due to Matt Taddy [?].

As an example, we fit the topic model for  $k=7$  on the Jaitin *et al* and we save the topic model output as a rda file under data folder.

```
StructureObj(t(fcounts),
             nclus_vec=7, tol=0.1,
             path_rda=" ../data/MouseJaitinSpleen-topicFit.rda")
```

We can also input a vector of clusters under *nclus\_vec*

```
StructureObj(t(deng.counts),
             nclus_vec=2:7, tol=0.1,
             path_rda=" ../data/MouseDeng2014-topicFit.rda")
```

```
StructureObj(t(gtex.counts),
             nclus_vec=4, tol=0.1,
             path_rda=" ../data/MouseDeng2014-topicFit.rda")
```

## 5 Structure plot visualization

---

Load the rda file

```
#data(MouseJaitinSpleen-topicFit)
MouseJaitinSpleen.topicFit <- readRDS("../data/MouseJaitinSpleen-topicFit.rds")
names(MouseJaitinSpleen.topicFit$clust_7)

## [1] "K"      "theta" "omega" "BF"     "D"      "X"

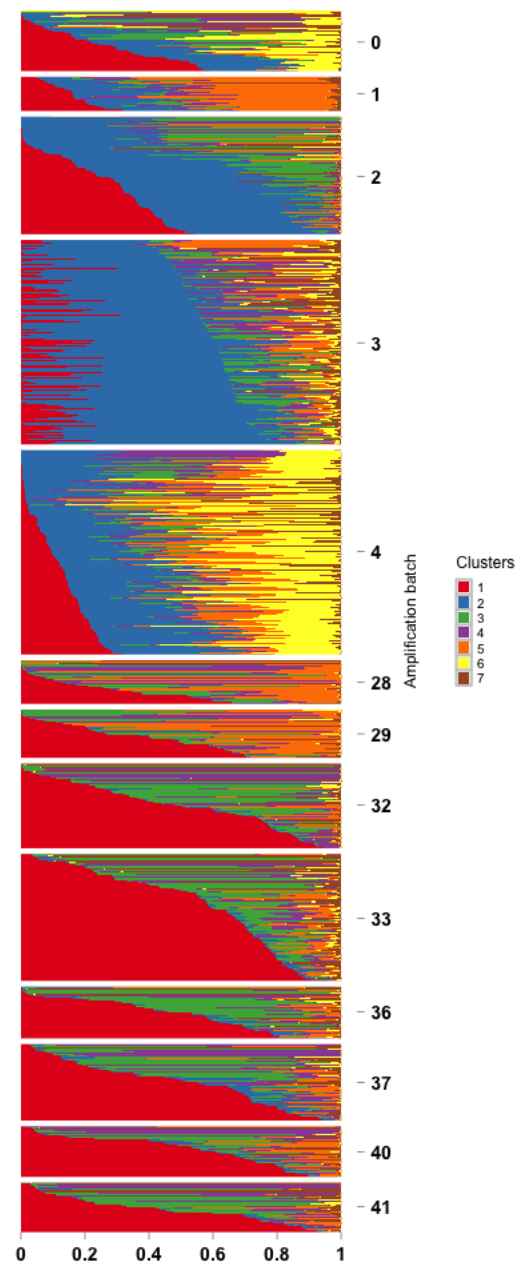
omega <- MouseJaitinSpleen.topicFit$clust_7$omega

amp_batch <- as.numeric(jaitin.meta_data_filtered[, "amplification_batch"])
annotation <- data.frame(
  sample_id = paste0("X", c(1:NROW(omega))),
  tissue_label = factor(amp_batch,
                        levels = rev(sort(unique(amp_batch)))) ) )
```

```

StructureGGplot(omega = omega,
  annotation = annotation,
  palette = RColorBrewer::brewer.pal(9, "Set1"),
  yaxis_label = "Amplification batch",
  order_sample = TRUE,
  axis_tick = list(axis_ticks_length = .1,
    axis_ticks_lwd_y = .1,
    axis_ticks_lwd_x = .1,
    axis_label_size = 7,
    axis_label_face = "bold"))

```



In the above plot, the samples within each batch is sorted by the proportion of representedness of the principal cluster driving it. One can use `order_sample=FALSE` to retain the order of the samples as in the data.

Now we perform the visualization for  $k=6$ .

```
MouseDeng2014.topicFit <- get(load("../data/MouseDeng2014-topicFit.rda"))
names(MouseDeng2014.topicFit$clust_6)
## [1] "K"      "theta" "omega" "BF"     "D"      "X"

omega <- MouseDeng2014.topicFit$clust_6$omega

annotation <- data.frame(
  sample_id = paste0("X", c(1:NROW(omega))),
  tissue_label = factor(rownames(omega),
                        levels = rev( c("zy", "early2cell",
                                         "mid2cell", "late2cell",
                                         "4cell", "8cell", "16cell",
                                         "earlyblast", "midblast",
                                         "lateblast") ) ) )

rownames(omega) <- annotation$sample_id;
```

Now we perform for GTEx V6 data for brain samples for  $k=4$ .

```
omega <- read.table("../data/omega_cis_genes_brain_2.txt",
                    header = TRUE, sep = " ",
                    stringsAsFactors = FALSE)

dim(omega)
## [1] 1259    4

colnames(omega) <- c(1:NCOL(omega))

tissue_labels <- gtex.meta_data[,3];

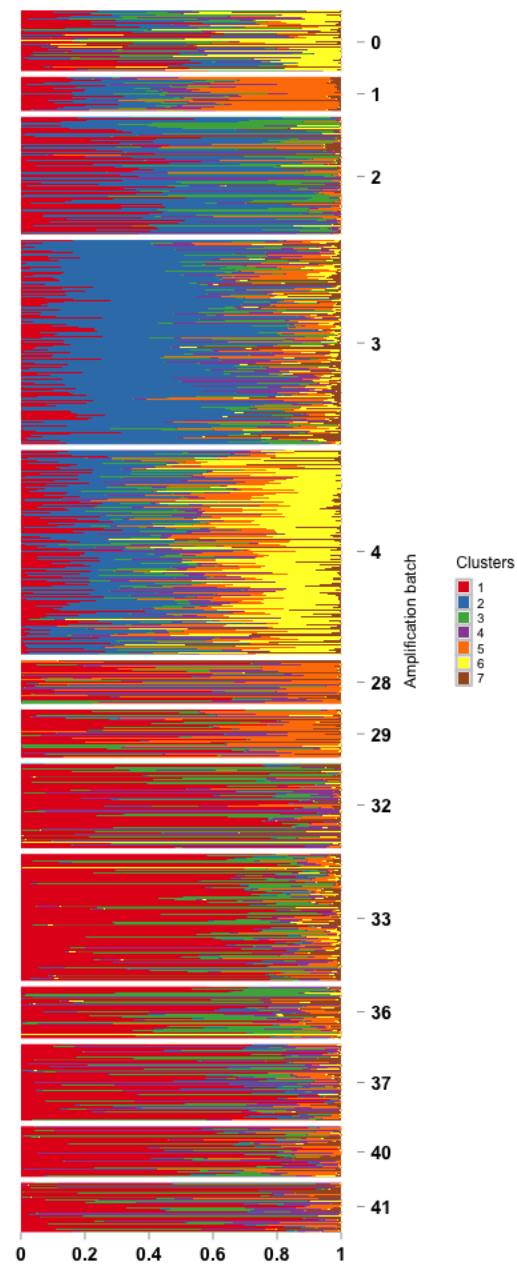
annotation <- data.frame(
  sample_id = paste0("X", 1:length(tissue_labels)),
  tissue_label = factor(tissue_labels,
                        levels = rev(unique(tissue_labels) ) ) );

cols <- c("blue", "darkgoldenrod1", "cyan", "red")
```

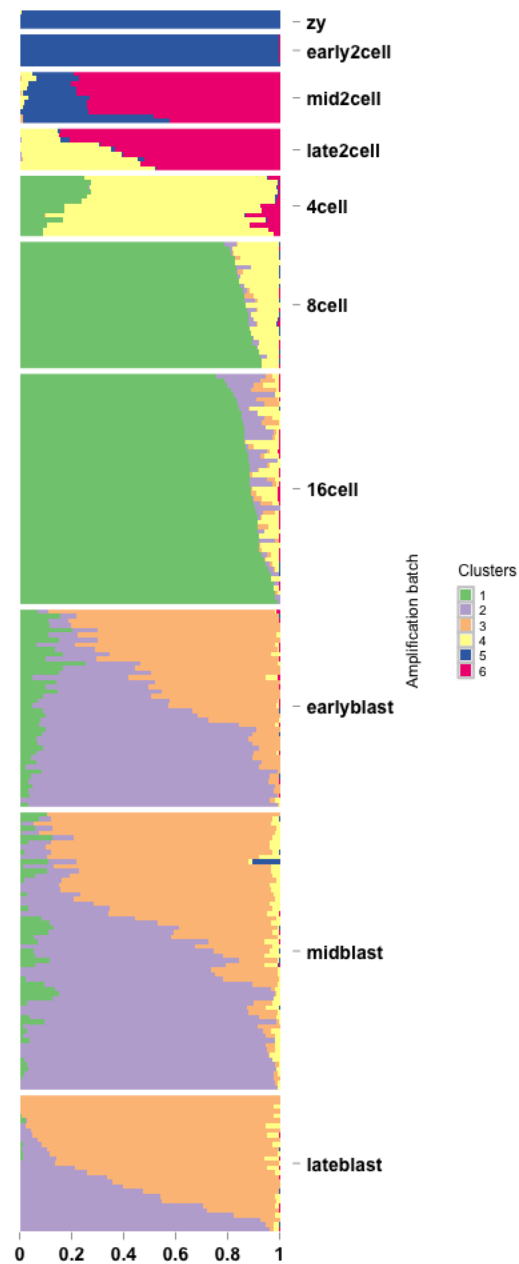
```

StructureGGplot(omega = omega,
  annotation = annotation,
  palette = RColorBrewer::brewer.pal(9, "Set1"),
  yaxis_label = "Amplification batch",
  order_sample = FALSE,
  axis_tick = list(axis_ticks_length = .1,
    axis_ticks_lwd_y = .1,
    axis_ticks_lwd_x = .1,
    axis_label_size = 7,
    axis_label_face = "bold"))

```



```
StructureGGplot(omega = omega,
  annotation = annotation,
  palette = RColorBrewer::brewer.pal(8, "Accent"),
  yaxis_label = "Amplification batch",
  order_sample = TRUE,
  axis_tick = list(axis_ticks_length = .1,
    axis_ticks_lwd_y = .1,
    axis_ticks_lwd_x = .1,
    axis_label_size = 7,
    axis_label_face = "bold"))
```

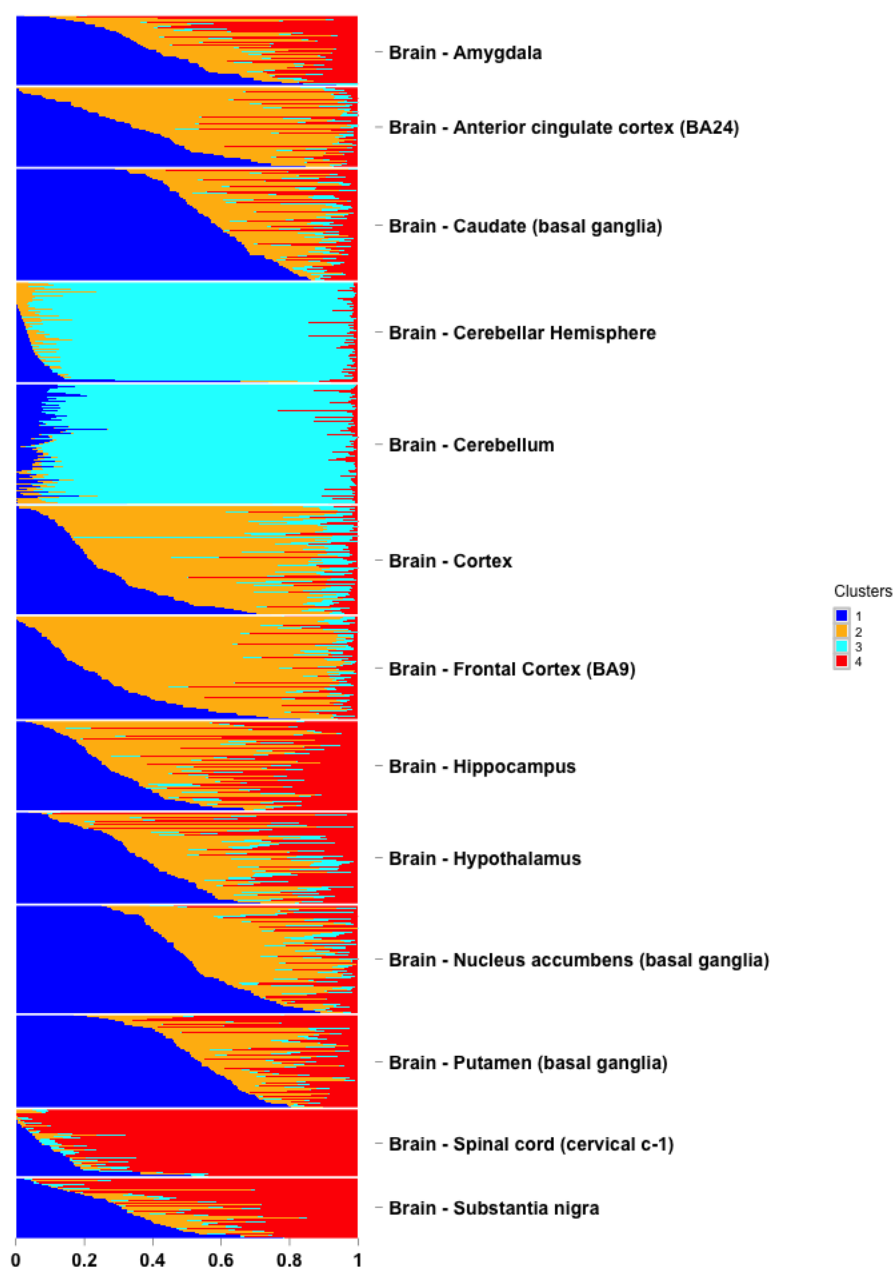




```

StructureGGplot(omega = omega,
  annotation= annotation,
  palette = cols,
  yaxis_label = "",
  order_sample = TRUE,
  split_line = list(split_lwd = .4,
    split_col = "white"),
  axis_tick = list(axis_ticks_length = .1,
    axis_ticks_lwd_y = .1,
    axis_ticks_lwd_x = .1,
    axis_label_size = 7,
    axis_label_face = "bold"))

```



## 6 Cluster Annotations

We extract the top genes driving each cluster using the `ExtractTopFeatures()` functionality of the *CountClust* package.

```
theta_mat <- MouseDeng2014.topicFit$clust_6$theta;
top_features <- ExtractTopFeatures(theta_mat, top_features=100,
                                   method="poisson", options="min");
gene_list <- do.call(rbind, lapply(1:dim(top_features)[1],
                                   function(x) deng.gene_names[top_features[x,]]))
```

We tabulate the top 5 genes for the 6 clusters.

```
library(xtable)
xtable(gene_list[,1:5])
```

	1	2	3	4	5
1	Timd2	Isyna1	Alpl2	Pramel5	Hsp90ab1
2	Upp1	Tdgf1	Aqp8	Fabp5	Tat
3	Actb	Krt18	Fabp3	Id2	Tspan8
4	Rtn2	Ebna1bp2	Zfp259	Nasp	Cenpe
5	LOC100502936	Bcl2l10	Tcl1	E330034G19Rik	Oas1d
6	Obox3	Zfp352	Gm8300	Usp17l5	BB287469

```
theta_mat <- as.matrix(read.table("../data/theta_cis_genes_brain_2.txt"));
top_features <- ExtractTopFeatures(theta_mat, top_features=100,
                                   method="poisson", options="min");
gene_list <- do.call(rbind, lapply(1:dim(top_features)[1],
                                   function(x) gtex.gene_names[top_features[x,]]))
```

The top 3 genes (ensemble IDs) driving the 4 clusters in the GTEx V6 brain data

```
library(xtable)
xtable(gene_list[,1:3])
```

	1	2	3
1	ENSG00000120885.15	ENSG00000130203.5	ENSG00000131771.9
2	ENSG00000171617.9	ENSG00000160014.12	ENSG00000154146.8
3	ENSG00000112139.10	ENSG00000139899.6	ENSG00000008710.13
4	ENSG00000197971.10	ENSG00000266844.1	ENSG00000237973.1

## 7 Supplementary analysis

## References

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- [1] Pritchard, Jonathan K., Matthew Stephens, and Peter Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155.2, 945-959.
- [2] Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA, Feldman MW. 2002. The genetic structure of human populations. *Science*. 298, 2381-2385.
- [3] Blei DM, Ng AY, Jordan MI. 2003. Latent Dirichlet Allocation. *J. Mach. Learn. Res.*. 3, 993-1022
- [4] Matt Taddy. 2012. On Estimation and Selection for Topic Models. *AISTATS 2012, JMLR W&CP* 22.(maptpx R package).
- [5] Jaitin DA, Kenigsberg E et al. 2014. Massively Parallel Single-Cell RNA-Seq for Marker-Free Decomposition of Tissues into Cell Types. *Science*. 343 (6172) 776-779.
- [6] Deng Q, Ramskold D, Reinius B, Sandberg R. 2014. Single-Cell RNA-Seq Reveals Dynamic, Random Monoallelic Gene Expression in Mammalian Cells. *Science*. 343 (6167) 193-196.
- [7] The GTEx Consortium. 2013. The Genotype-Tissue Expression (GTEx) project. *Nature genetics*. 45(6): 580-585. doi:10.1038/ng.2653.

## 8 Session Info

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```
sessionInfo()

## R version 3.2.2 (2015-08-14)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.10.5 (Yosemite)
##
## 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] parallel stats      graphics grDevices utils      datasets  methods
##  [8] base
##
## other attached packages:
##  [1] cowplot_0.6.0
##  [2] reshape2_1.4.1
##  [3] ggplot2_2.0.0
##  [4] GTExV6Brain_0.0.1
##  [5] singleCellRNASeqMouseDeng2014_0.99.0
##  [6] singleCellRNASeqMouseJaitinSpleen_0.99.0
##  [7] Biobase_2.30.0
##  [8] BiocGenerics_0.16.1
```

```
## [9] CountClust_0.1.0
## [10] knitr_1.12.3
##
## loaded via a namespace (and not attached):
## [1] Rcpp_0.12.3      digest_0.6.9     plyr_1.8.3       grid_3.2.2
## [5] gtable_0.1.2     formatR_1.2.1    magrittr_1.5     scales_0.3.0
## [9] evaluate_0.8     highr_0.5.1      stringi_1.0-1    labeling_0.3
## [13] BiocStyle_1.8.0  RColorBrewer_1.1-2 tools_3.2.2      stringr_1.0.0
## [17] munsell_0.4.2    colorspace_1.2-6
```