Generating heatmaps for Nonnegative Matrix Factorization Package NMF - Version 0.9

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Abstract

This vignette describes how to produce different informative heatmaps from NMF objects, such as returned by the function nmf in the NMF package¹ (Gaujoux et al. 2010). The main drawing engine is implemented by the function aheatmap, which is a highly enhanced modification of the function pheatmap from the *pheatmap* package² (Kolde 2012), and provides convenient and quick ways of producing high quality and customizable annotated heatmaps. Currently this function is part of the package NMF, but may eventually compose a separate package on its own.

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1 Preliminaries

1.1 Quick reminder on NMF models

Given a nonnegative target matrix X of dimension $n \times p$, NMF algorithms aim at finding a rank k approximation of the form:

 $X \approx WH$.

where W and H are nonnegative matrices of dimensions $n \times k$ and $k \times p$ respectively.

The matrix W is the basis matrix, whose columns are the basis components. The matrix H is the mixture coefficient or weight matrix, whose columns contain the contribution of each basis component to the corresponding column of X. We call the rows of H the basis profiles.

1.2 Heatmaps for NMF

Because NMF objects essentially wrap up a pair of matrices, heatmaps are convenient to visualise the results of NMF runs. The package NMF provides several specialised heatmap functions, designed to produce heatmaps with sensible default configurations according to the data being drawn. Being all based on a common drawing engine, they share almost identical interfaces and capabilities. The following specialised functions are currently implemented:

¹http://cran.r-project.org/package=NMF

²http://cran.r-project.org/package=pheatmap

basismap draws heatmaps of the basis matrix

coefmap draws heatmaps of the mixture coefficient matrix

consensusmap draws heatmaps of the consensus matrix, for results of multiple NMF runs.

1.3 Heatmap engine

All the above functions eventually call a common heatmap engine, with different default parameters, chosen to be relevant for the given underlying data. The engine is implemented by the function aheatmap. Its development started as modification of the function pheatmap from the pheatmap package. The initial objective was to improve and increase its capabilities, as well as defining a simplified interface, more consistent with the R core function heatmap. We eventually aim at providing a general, flexible, powerful and easy to use engine for drawing annotated heatmaps.

The function aheatmap has many advantages compared to other heatmap functions such as heatmap, gplots::heatmap2, heatmap.plus::heatmap.plus , or even pheatmap:

- Annotations: unlimited number of annotation tracks can be added to *both* columns and rows, with automated colouring for categorical and numeric variables.
- Compatibility with both base and grid graphics: the function can be directly called in drawing contexts such as grid, mfrow or layout. This is a feature many R users were looking for, and that was strictly impossible with base heatmaps.
- Legends: default automatic legend and colouring;
- Customisation: clustering methods, annotations, colours and legend can all be customised, even separately for rows and columns;
- Convenient interface: many arguments provide multiple ways of specifying their value(s), which speeds up developping/writing and reduce the amount of code required to generate customised plots (e.g. see ??).
- Aesthetics: the heatmaps look globally cleaner, the image and text components are by default well proportioned relatively to each other, and all fit within the graphic device.

1.4 Data and model

For the purpose of illustrating the use of each heatmap function, we generate a random target matrix, as well as some annotations or covariates:

```
# random data that follow an 3-rank NMF model (with quite some noise:
# sd=2)
X <- syntheticNMF(100, 3, 20, noise = 2)

# row annotations and covariates
n <- nrow(X)
d <- rnorm(n)
e <- unlist(mapply(rep, c("X", "Y", "Z"), 10))
e <- c(e, rep(NA, n - length(e)))
rdata <- data.frame(Var = d, Type = e)

# column annotations and covariates
p <- ncol(X)
a <- sample(c("alpha", "beta", "gamma"), p, replace = TRUE)</pre>
```

```
# define covariates: true groups and some numeric variable
c <- rnorm(p)
# gather them in a data.frame
covariates <- data.frame(a, X$pData, c)</pre>
```

Note that in the code above, the object X returned by syntheticNMF really is a matrix object, but wrapped through the function ExposedAttribute object, which exposes its attributes via a more friendly and access controlled interface \$. Of particular interests are attributes 'pData' and 'fData', which are lists that contain a factor named 'Group' that indicates the true underlying clusters. These are respectively defined as each sample's most contributing basis component and the basis component to which each feature contributes the most. They are useful to annotate heatmaps and assess the ability of NMF methods to recover the true clusters.

As an example, one can conveniently visualize the target matrix as a heatmap, with or without the relevant sample and feature annotations, using simple calls to the aheatmap function:

```
par(mfrow = c(1, 2))
aheatmap(X, annCol = covariates, annRow = X$fData)
aheatmap(X)
```

Then, we fit an NMF model using multiple runs, that will be used throughtout this vignette to illustrate the use of NMF heatmaps:

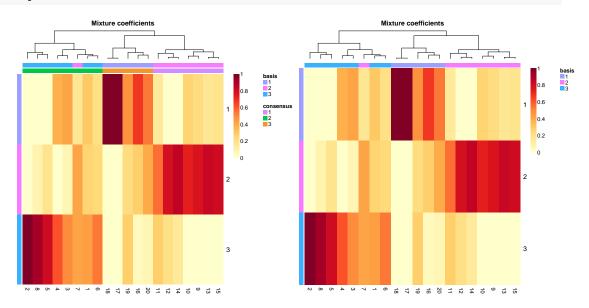
```
res \leftarrow nmf(X, 3, nrun = 10)
res
## <Object of class: NMFfitX1 >
##
     Method: brunet
##
     Runs: 10
     RNG:
##
      407L, 1836648330L, -690549885L, 21815016L, -792180791L, -1546669834L, 1782788863L
##
##
     Total timing:
##
      user system elapsed
##
     2.556 0.092 3.810
```

NB: To keep the vignette simple, we always use the default NMF method (i.e. 'brunet'), but all steps could be performed using a different method, or multiple methods in order to compare their perfromances.

2 Mixture Coefficient matrix: coefmap

The coefficient matrix of the result can be plotted using the function coefmap. The default behaviour for multiple NMF runs is to add two annotation tracks that show the clusters obtained by the best fit and the hierarchical clustering of the consensus matrix³. In the legend, these tracks are named *basis* and *consensus* respectively. For single NMF run or NMF model objects, no consensus data are available, and only the clusters from the fit are displayed.

```
opar <- par(mfrow = c(1, 2))
# coefmap from multiple run fit: includes a consensus track
coefmap(res)
# coefmap of a single run fit: no consensus track
coefmap(minfit(res))
par(opar)</pre>
```



NB: Note how both heatmaps were drawn on the same plot, simply using the standard call to par(mfrow=c(1,2). This is impossible to achieve with the R core function heatmap. See Section 5 for more details about compatibility with base and grid graphics.

By default:

- the rows are not ordered;
- the columns use the default ordering of aheatmap, but may easily be ordered according to the clusters defined by the dominant basis component for each column with Colv="basis",

 $^{^3}$ The hierarchical clustering is computed using the consensus matrix itself as a similarity measure, and average linkage. See ?consensushc.

or according to those implied by the consensus matrix, i.e. as in consensusmap, with Colv="consensus";

- each column is scaled to sum up to one;
- the color palette used is 'YlOrRd' from the *RColorBrewer* package⁴ (Neuwirth 2011), with 50 breaks.

In term of arguments passed to the heatmap engine aheatmap, these default settings translate as:

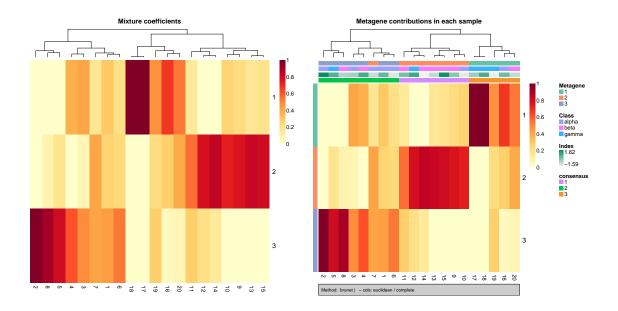
```
Rowv = NA
Colv = TRUE
scale = "c1"
color = "Y10rRd:50"
annCol = predict(object) + predict(object, "consensus")
```

If the ordering does not come from a hierarchical clustering (e.g., if Colv='basis'), then no dendrogram is displayed. The default behaviour of aheatmap can be obtained by setting arguments Rowv=TRUE, Colv=TRUE, scale='none'.

The automatic annotation tracks can be hidden all together by setting argument tracks=NA, displayed separately by passing only one of the given names (e.g. tracks=':basis' or tracks='basis' for the row or column respectively), and their legend names may be changed by specifying e.g. tracks=c(Metagene=':basis', 'consensus'). Beside this, they are handled by the heatmap engine function aheatmap and can be customised as any other annotation tracks – that can be added via the same argument annCol (see Section 5 or ?aheatmap for more details).

```
opar <- par(mfrow=c(1,2))
# removing all automatic annotation tracks
coefmap(res, tracks=NA)
# customized plot
coefmap(res, Colv = 'euclidean'
, main = "Metagene contributions in each sample", labCol = NULL
, annRow = list(Metagene=':basis'), annCol = list(':basis', Class=a, Index=c)
, annColors = list(Metagene='Set2')
, info = TRUE)
par(opar)</pre>
```

⁴http://cran.r-project.org/package=RColorBrewer



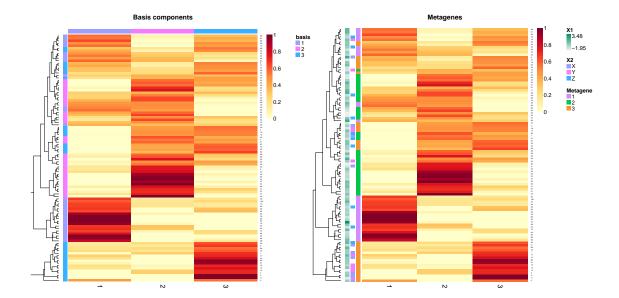
NB: The feature that allows to display some information about the fit at the bottom of the plot via argument info=TRUE is still experimental. It is helpful mostly when developing algorithms or doing an analysis, but would seldom be used in publications.

3 Basis matrix: basismap

The basis matrix can be plotted using the function basismap. The default behaviour is to add an annotation track that shows for each row the dominant basis component. That is, for each row, the index of the basis component with the highest loading.

This track can be disabled by setting tracks=NA, and extra row annotations can be added using the same argument annRow.

```
opar <- par(mfrow = c(1, 2))
# default plot
basismap(res)
# customized plot: only use row special annotation track.
basismap(res, main = "Metagenes", annRow = list(d, e), tracks = c(Metagene = ":basis"))
par(opar)</pre>
```



By default:

- the columns are not ordered;
- the rows are ordered by hierarchical clustering using default distance and linkage methods ('eculidean' and 'complete');
- each row is scaled to sum up to one;
- the color palette used is 'YlOrRd' from the *RColorBrewer* package⁵ (Neuwirth 2011), with 50 breaks.

In term of arguments passed to the heatmap engine aheatmap, these default settings translate as:

```
Colv = NA
scale = "r1"
color = "YlOrRd:50"
annRow = predict(object, "features")
```

4 Consensus matrix: consensusmap

When doing clustering with NMF, a common way of assessing the stability of the clusters obtained for a given rank is to consider the consensus matrix computed over multiple independent NMF runs, which is the average of the connectivity matrices of each separate run ⁶. This procedure is usually repeated over a certain range of factorization ranks, and the results are compared to identify which rank gives the best clusters, possibly in the light of some extra knowledge one could have about the samples (e.g. covariates). The functions nmf and consensusmap make it easy to implement this whole process.

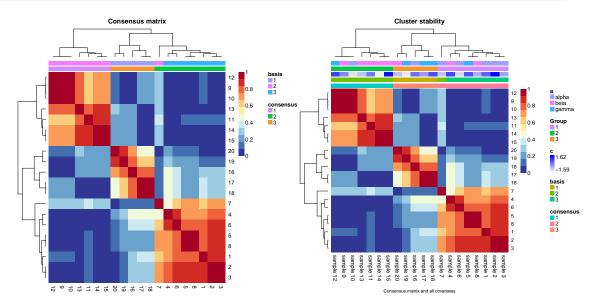
 $^{^{5} \}rm http://cran.r-project.org/package=RColorBrewer$

⁶Hence, stability here means robustness with regards to the initial starting point, and shall not be interpreted as in e.g. cross-validation/bootstrap analysis. However, one can argue that having very consistent clusters across runs somehow supports for a certain regularity or the presence of an underlying pattern in the data.

NB: The consensus plots can also be generated for fits obtained from single NMF runs, in which case the consensus matrix simply reduces to a single connectivity matrix. This is a binary matrix (i.e. entries are either 0 or 1), that will always produce a bi-colour heatmap, and by default clear blocks for each cluster.

4.1 Single fit

In section Section 1.4, the NMF fit res was computed with argument nrun=10, and therefore contains the best fit over 10 runs, as well as the consensus matrix computed over all the runs ⁷. This can be plotted using the function consensusmap, which allows for the same kind of customization as the other NMF heatmap functions:



By default:

- the rows and columns of the consensus heatmap are symmetrically ordered by hierarchical clustering using the consensus matrix as a similarity measure and average linkage, and the associated dendrogram is displayed;
- the color palette used is the reverse of 'RdYlBu' from the *RColorBrewer* package⁸ (Neuwirth 2011).

In term of arguments passed to the heatmap engine aheatmap, these default settings translate as:

⁷If one were interested in keeping the fits from all the runs, the function nmf should have been called with argument .options='k'. See section *Options* in ?nmf. The downstream handling of the result would remain identical.

⁸http://cran.r-project.org/package=RColorBrewer

```
distfun = function(x) as.dist(1 - x) # x being the consensus matrix
hclustfun = "average"
Rowv = TRUE
Colv = "Rowv"
color = "-RdYlBu"
```

4.2 Single method over a range of ranks

The function nmf accepts a range of value for the rank (argument rank), making it fit NMF models for each value in the given range 9:

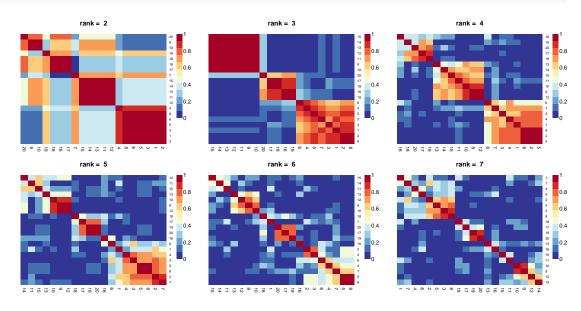
```
res2_7 <- nmf(X, 2:7, nrun = 10, .options = "v")

## Compute NMF rank= 2 ... + measures ... OK
## Compute NMF rank= 3 ... + measures ... OK
## Compute NMF rank= 4 ... + measures ... OK
## Compute NMF rank= 5 ... + measures ... OK
## Compute NMF rank= 6 ... + measures ... OK
## Compute NMF rank= 7 ... + measures ... OK
class(res2_7)

## [1] "NMF.rank"</pre>
```

The result res2_7 is an S3 object of class 'NMF.rank', that contains — amongst other data — a list of the best fits obtained for each value of the rank in range [2,7]]. The method of consensusmap defined for class 'NMF.rank', which plots all the consensus matrices on the same plot:

consensusmap(res2_7)



⁹Before version 0.6, this feature was provided by the function nmfEstimateRank. From version 0.6, the function nmf accepts ranges of ranks, and internally calls the function nmfEstimateRank – that remains exported and can still be called directly. See documentation ?nmfEstimateRank for more details on the returned value.

NB: The main title of each consensus heatmap can be customized by passing to argument main a character vector or a list whose elements specify each title. All other arguments are used in each internal call to consensusmap, and will therefore affect all the plots simultaneously. The layout can be specified via argument layout as a numeric vector giving the number of rows and columns in a mfrow-like way, or as a matrix that will be passed to R core function layout. See ?consensusmap for more details and example code.

4.3 Single rank over a range of methods

If one is interested in comparing methods, for a given factorization rank, then on can fit an NMF model for each method by providing the function nmf with a list in argument method:

```
res_methods <- nmf(X, 3, list("lee", "brunet", "nsNMF"), nrun = 10)

## Compute NMF method 'lee' [1/3] ... OK

## Compute NMF method 'brunet' [2/3] ... OK

## Compute NMF method 'nsNMF' [3/3] ... OK

class(res_methods)

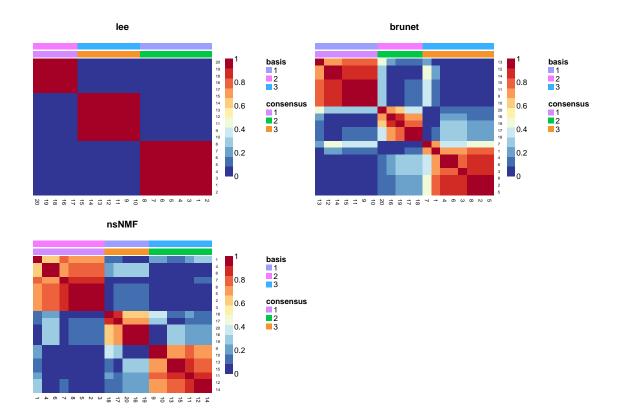
## [1] "NMFList"

## attr(,"package")

## [1] "NMF"</pre>
```

The result res_methods is an S4 object of class NMFList, which is essentially a named list, that contains each fits and the CPU time required by the whole computation. As previously, the sequence of consensus matrices is plotted with consensusmap:

```
consensusmap(res_methods)
```



5 Generic heatmap engine: aheatmap

This section still needs to be written, but many examples of annotated heatmaps can be found in the demos 'aheatmap' and 'heatmaps':

```
demo("aheatmap")
# or
demo("heatmaps")
```

These demos and the plots they generate can also be browsed online at http://nmf.r-forge.r-project.org/_DEMOS.html.

6 Session Info

- R version 2.15.2 (2012-10-26), i686-pc-linux-gnu
- Locale: LC_CTYPE=en_ZA.UTF-8, LC_NUMERIC=C, LC_TIME=en_ZA.UTF-8, LC_COLLATE=en_ZA.UTF-8, LC_MONETARY=en_ZA.UTF-8, LC_MESSAGES=en_ZA.UTF-8, LC_PAPER=C, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_ZA.UTF-8, LC_IDENTIFICATION=C
- Base packages: base, datasets, graphics, grDevices, grid, methods, parallel, stats, utils
- Other packages: bigmemory 4.3.0, bigmemory.sri 0.1.2, Biobase 2.16.0, BiocGenerics 0.2.0, codetools 0.2-8, colorspace 1.2-0, digest 0.5.2, doParallel 1.0.1, foreach 1.4.0, gridBase 0.4-5, iterators 1.0.6, knitr 0.8, NMF 0.9, pkgmaker 0.9.2, RColorBrewer 1.0-5, registry 0.2, rngtools 1.0, stringr 0.6.1

 \bullet Loaded via a name space (and not attached): evaluate 0.4.2, format R 0.6, plyr 1.7.1, tools 2.15.2, xtable 1.7-0

References

- [1] Renaud Gaujoux and Cathal Seoighe. "A flexible R package for nonnegative matrix factorization". In: *BMC Bioinformatics* 11.1 (2010), p. 367. ISSN: 1471-2105. DOI: 10.1186/1471-2105-11-367. URL: http://www.biomedcentral.com/1471-2105/11/367 (see p. 1).
- [2] Raivo Kolde. *pheatmap: Pretty Heatmaps*. R package version 0.7.3. 2012. URL: http://CRAN. R-project.org/package=pheatmap (see p. 1).
- [3] Erich Neuwirth. *RColorBrewer: ColorBrewer palettes*. R package version 1.0-5. 2011. URL: http://CRAN.R-project.org/package=RColorBrewer (see pp. 5, 7, 8).