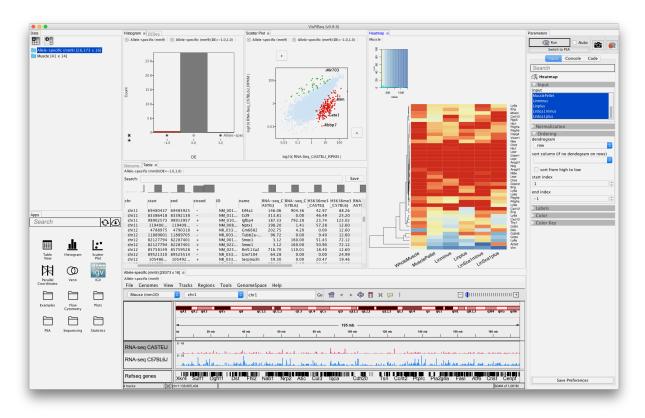
Making your packages accessible to non-programmer collaborators using the VisRseq framework

Hamid Younesy June 26, 2016 | BioC 2016

Abstract

I will introduce the VisRseq framework and walk the participants through the quick process of creating modules called R-apps from R packages. I expect this to be specially useful for bioinformaticians and package developers that develop R-based analysis tools and would like to make them accessible to their non-programmer collaborators or to the public without having to spend significant time on creating extensive graphical user interfaces. I will walk the participants through several examples of creating diverse types of apps, from simple plotting (e.g. plots) to intermediate (e.g. clustering) to more advanced (e.g. edgeR and DEseq) packages. I will also show how several R-apps can be linked together to create more complex workflows. Participants will require having beginner/intermediate knowledge of R and a machine with R and Java installation.

VisRseq Overview



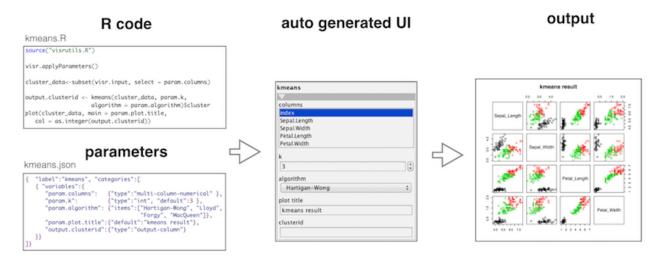
Goals for this workshop

- Learn about VisRseq framework.
- Learn how to create R-apps through several examples.

Requirements

To get the most out of this workshop you are recommended to install the latest version of VisRseq from visrseq.github.io/downloads. You can still follow the instructions and create R-apps using plain R, however you will require VisRseq to be able to run the apps and see the results.

Structure of an R-App



To create an R app you need a .R code containing the main script together with a .json file specifying parameters. Each R app can have any number of "parameters", grouped into "categories". The .json file can be created manually or using the helper function in visrutils.R

```
source("https://raw.githubusercontent.com/hyounesy/visr-apps/master/visrutils.R")
```

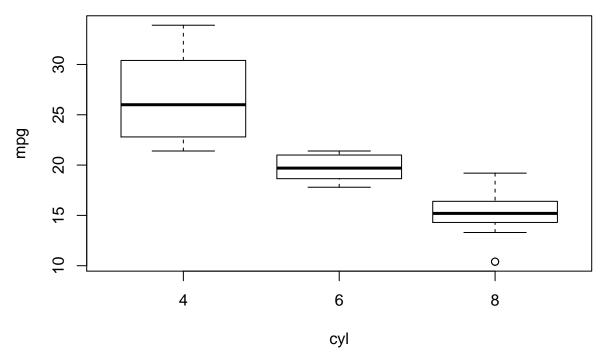
which defines the following functions among other things.

Let's get started and create our first app!

Example 1: Boxplot

Let's start simple by creating a basic app that draws a barplot. Let's first write a basic boxplot script in a file simple_boxplot.R:





Assume we would like to allow user to use their own data set and be able to specify x and y columns from their data.

We start the app definition by calling visr.app.start and specifying the app name. If we want be able to debug this app in R, we pass a test data set to debugdata. This parameter will be ignored when running the app in VisRseq. The data assigned to an app will be stored in visr.input variable.

```
visr.app.start("Simple Boxplot", debugdata=mtcars)
```

We then add two parameters: y the numeric vector of data values that will be split into groups according to the parameter group. The parameter y has to be a numerical column in the input data, so we specify the type="column-numerical". The parameter group can be any column, so we specify type="column". In order to be able the debug the app inside the R, we also specify the values using debugvalue. This is optional but recommended.

```
visr.param("y", type="column-numerical", debugvalue="mpg")
visr.param("group", type="column", debugvalue="cyl")
```

This will create two parameters visr.param.y and visr.param.group. When running the script in R, they will be initialized with their corresponding debugvalue column names. When running the app in VisRseq, they will contain the string name of the column selected in the GUI.

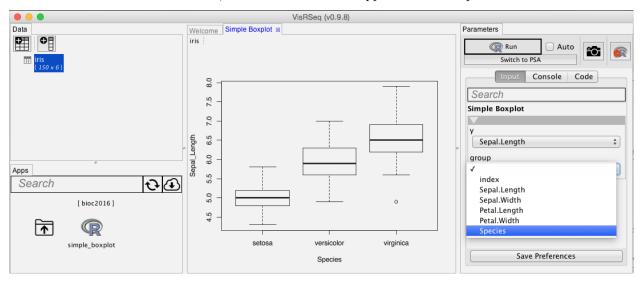
We end the definition by calling visr.app.end. If argument printjson == TRUE, the json data for the app parameters will be printed to console. If argument writefile == TRUE, the output .json file will be written to a file specified by filename argument. If filename is not specified, the json file will be created at the same path of the R script, and same name but .json extension (i.e. simple_boxplot.json).

```
visr.app.end(printjson=TRUE, writefile = FALSE, filename = NULL)
```

```
## {
     "label": "Simple Boxplot",
##
     "info": "",
##
     "categories":[ {
##
##
       "label": "",
       "info": "",
##
##
       "variables": {
          "visr.param.y": {
##
##
            "type": "column-numerical"
##
         },
##
         "visr.param.group": {
            "type": "column"
##
##
##
##
     }]
## }
```

We now have the json file. So we just have to modify our original R script to use the correct parameter names.

Done! now if we place the simple_boxplot.R and simple_boxplot.json inside VisRseq/visr/srcR/bioc2016 and hit the Refresh icon above the apps pane, we will have our first custom app in VisRseq under the bioc2016 folder. For demonstration, here we have used the app to draw a boxplot for the iris dataset.



Below is the complete source code for simple_boxplot.R app:

```
source("visrutils.R")
# parameters
visr.app.start("Simple Boxplot", debugdata=mtcars)
visr.param("y", type="column-numerical", debugvalue="mpg")
visr.param("group", type="column", debugvalue="cyl")
visr.app.end(printjson=TRUE, writefile = FALSE, filename = NULL)
```

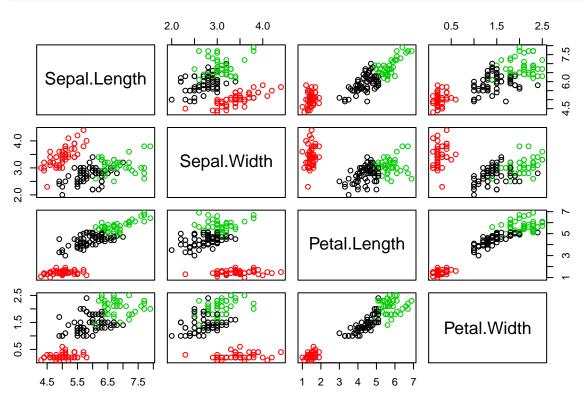
Exercise: Violin Plot

Let's do an exercise and create another app to draw violin plots.

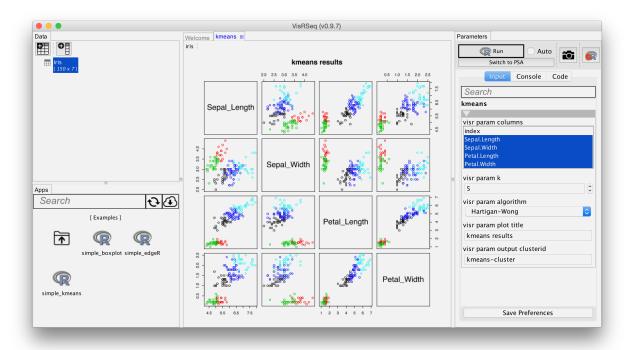
Example 2: Kmeans

We will now make something more interesting: A kmeans clustering app. Like the previous example, we will start with the R script for kmeans clustering.

```
input <- iris
columns <- c("Sepal.Length", "Sepal.Width", "Petal.Length", "Petal.Width")
cluster_data<-subset(input, select = columns)
clusterid <- kmeans(cluster_data, centers = 3)$cluster
plot(cluster_data, col = as.integer(clusterid))</pre>
```



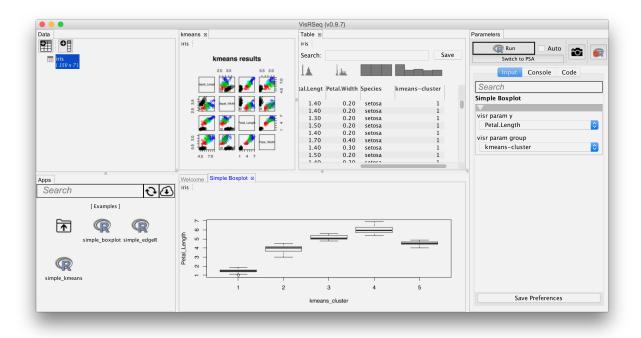
```
visr.param("k", default = 3)
visr.param("algorithm", items = c("Hartigan-Wong", "Lloyd", "Forgy", "MacQueen"))
visr.category("output")
visr.param("plot.title", default = "kmeans results")
visr.param("output.clusterid", type = "output-column")
visr.app.end(printjson=TRUE)
## {
##
     "label": "Simple Kmeans",
     "info": "",
##
##
     "categories":[ {
       "label": "clustering parameters",
##
##
       "info": "",
##
       "variables": {
##
         "visr.param.columns": {
           "type": "multi-column-numerical"
##
##
##
         "visr.param.k": {
##
           "type": "int",
           "default": 3
##
##
         },
##
         "visr.param.algorithm": {
           "type": "string",
##
##
           "items": [ "Hartigan-Wong", "Lloyd", "Forgy", "MacQueen" ]
##
         }
##
       }
##
     },
##
##
       "label": "output",
##
       "info": "",
##
       "variables": {
##
         "visr.param.plot.title": {
           "type": "string",
##
           "default": "kmeans results"
##
##
##
         "visr.param.output.clusterid": {
           "type": "output-column"
##
##
##
##
     }]
## }
visr.applyParameters()
cluster_data<-subset(visr.input, select = visr.param.columns)</pre>
visr.param.output.clusterid <- kmeans(cluster_data,</pre>
                                        centers = visr.param.k,
                                        algorithm = visr.param.algorithm)$cluster
plot(cluster_data, main = visr.param.plot.title,
     col = as.integer(visr.param.output.clusterid))
```



Below is the complete source code for simple_kmeans.R app:

```
source("visrutils.R")
# parameters
visr.app.start("Simple Kmeans", debugdata = iris)
visr.category("clustering parameters")
visr.param("columns", type = "multi-column-numerical",
           debugvalue = c("Sepal.Length", "Sepal.Width", "Petal.Length", "Petal.Width"))
visr.param("k", default = 3)
visr.param("algorithm", items = c("Hartigan-Wong", "Lloyd", "Forgy", "MacQueen"))
visr.category("output")
visr.param("plot.title", default = "kmeans results")
visr.param("output.clusterid", type = "output-column")
visr.app.end(printjson=TRUE)
visr.applyParameters()
# kmeans code
cluster_data<-subset(visr.input, select = visr.param.columns)</pre>
visr.param.output.clusterid <- kmeans(cluster data,</pre>
                                       centers = visr.param.k,
                                       algorithm = visr.param.algorithm)$cluster
plot(cluster_data, main = visr.param.plot.title,
     col = as.integer(visr.param.output.clusterid))
```

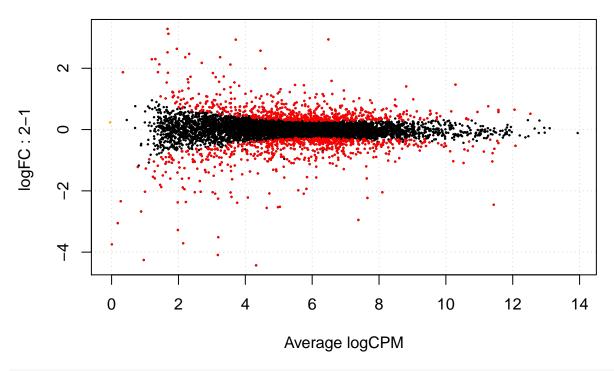
Chaining apps When running kmeans inside VisRseq on a test data, the result of the clustering will be stored in a new column specified by output cluster id. We can preview this new column in a table view app, or even better we can now use that with the boxplot app we created earlier.



Example 3: Differntial expression analysis using edgeR

```
library("edgeR", quietly=T)
countdata = read.table(
  "https://raw.githubusercontent.com/hyounesy/bioc2016.visrseq/master/data/counts.txt",
 header=T, row.names = 1)
x <- countdata
head(x)
##
                CT.PA.1 CT.PA.2 KD.PA.3 KD.PA.4 CT.SI.5 KD.SI.6 CT.SI.7
## FBgn0000008
                     76
                             71
                                      87
                                              68
                                                      137
                                                                        82
                                                              115
## FBgn0000017
                   3498
                           3087
                                    3029
                                            3264
                                                     7014
                                                             4322
                                                                      3926
## FBgn0000018
                            306
                                             307
                    240
                                     288
                                                      613
                                                              528
                                                                       485
## FBgn0000032
                    611
                            672
                                     694
                                             757
                                                     1479
                                                             1361
                                                                      1351
## FBgn0000042
                  40048
                          49144
                                   70574
                                           72850
                                                    97565
                                                            95760
                                                                     99372
## FBgn0000043
                  15910
                          18194
                                   31086
                                           34085
                                                            42389
                                                    34171
                                                                     29671
group1 <- c("CT.PA.1", "CT.PA.2")
group2 <- c("KD.PA.3", "KD.PA.4")</pre>
groups <- factor(c(rep(1, length(group1)), rep(2, length(group2)))) # c(1, 1, 2, 2)
# create edgeR's container for RNA-seq count data
y <- DGEList(counts=x[, c(group1, group2)], group = groups)
# estimate normalization factors
y <- calcNormFactors(y)</pre>
# estimate tagwise dispersion (simple design)
y <- estimateCommonDisp(y)</pre>
y <- estimateTagwiseDisp(y)</pre>
# test for differential expression using classic edgeR approach
et <- exactTest(y)</pre>
```

```
# total number of DE genes in each direction
is.de <- decideTestsDGE(et, adjust.method = "BH", p.value = 0.05, lfc = 0)
# The log-fold change for each gene is plotted against the average abundance
plotSmear(y, de.tags=rownames(y)[is.de!=0])</pre>
```

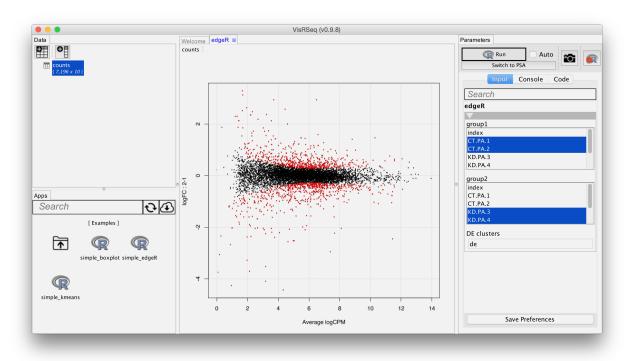


```
## edgeR parameters
visr.app.start("edgeR", debugdata = countdata)
visr.param("group1", type = "multi-column-numerical", debugvalue = c("CT.PA.1", "CT.PA.2"))
visr.param("group2", type = "multi-column-numerical", debugvalue = c("KD.PA.3", "KD.PA.4"))
visr.param("output.de", label = "DE clusters", type = "output-column")
visr.app.end(printjson=TRUE, writefile=T)
```

```
## {
##
     "label": "edgeR",
##
     "info": "",
##
     "categories":[ {
       "label": "",
##
       "info": "",
##
##
       "variables": {
         "visr.param.group1": {
##
           "type": "multi-column-numerical"
##
##
         "visr.param.group2": {
##
           "type": "multi-column-numerical"
##
##
         "visr.param.output.de": {
##
           "label": "DE clusters",
##
##
           "type": "output-column"
##
##
     }]
##
```

```
visr.applyParameters()
```

```
## edgeR code
library("edgeR")
x <- visr.input
groups <- factor(c(rep(1, length(visr.param.group1)), # e.q. 1,1</pre>
                    rep(2, length(visr.param.group2)))) # e.g. 2,2
# create edgeR's container for RNA-seq count data
y <- DGEList(counts=x[, c(visr.param.group1, visr.param.group2)], group = groups)
# estimate normalization factors
y <- calcNormFactors(y)</pre>
# estimate tagwise dispersion (simple design)
y <- estimateCommonDisp(y)</pre>
y <- estimateTagwiseDisp(y)</pre>
# test for differential expression using classic edgeR approach
et <- exactTest(y)</pre>
# total number of DE genes in each direction
is.de <- decideTestsDGE(et, adjust.method = "BH", p.value = 0.05, lfc = 0)</pre>
# export the results to VisRseq
visr.param.output.de <- as.factor(is.de)</pre>
# The log-fold change for each gene is plotted against the average abundance
plotSmear(y, de.tags = rownames(y)[is.de != 0])
```



Below is the complete source code for simple_edgeR.R app:

```
source("visrutils.R")
## edgeR parameters
```

```
visr.app.start("edgeR", debugdata = countdata)
visr.param("group1", type = "multi-column-numerical", debugvalue = c("CT.PA.1", "CT.PA.2"))
visr.param("group2", type = "multi-column-numerical", debugvalue = c("KD.PA.3", "KD.PA.4"))
visr.param("output.de", label = "DE clusters", type = "output-column")
visr.app.end(printjson=TRUE, writefile=T)
visr.applyParameters()
## edgeR code
library("edgeR")
x <- visr.input
groups <- factor(c(rep(1, length(visr.param.group1)), # e.g. 1,1</pre>
                   rep(2, length(visr.param.group2)))) # e.q. 2,2
# create edgeR's container for RNA-seq count data
y <- DGEList(counts=x[, c(visr.param.group1, visr.param.group2)], group = groups)
# estimate normalization factors
y <- calcNormFactors(y)</pre>
# estimate tagwise dispersion (simple design)
y <- estimateCommonDisp(y)</pre>
y <- estimateTagwiseDisp(y)</pre>
# test for differential expression using classic edgeR approach
et <- exactTest(y)</pre>
# total number of DE genes in each direction
is.de <- decideTestsDGE(et, adjust.method = "BH", p.value = 0.05, lfc = 0)</pre>
# export the results to VisRseq
visr.param.output.de <- as.factor(is.de)</pre>
# The log-fold change for each gene is plotted against the average abundance
plotSmear(y, de.tags = rownames(y)[is.de != 0])
```