Visualization and evaluation of RNA-seq data normalization – the Normalization Visualization Tool

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Abstract

Differential expression analysis, between two samples, is a common task in the analysis of RNA-Seq data. In order to identify differential expressed genes it is crucial that the two compared data sets are normalized. For the task of normalizing gene expression data there are multiple methods available. But all of them are based on certain assumptions that can or can not be meet by the data which should be normalized. An important question is, how the normalization affects the data and which normalization method and its assumptions blend well with the expression data. The *NVT* package provides a fast and simple way to analyze multiple normalization methods via visualization and correlation values. This vignette explains the use of the package and demonstrates a typical work flow.

NVT version: 0.3

Contents

1   Installation

Download the latest NVT package from: https://github.com/NexusX/NVT/releases and install it with the following command.

```
install.packages(file.path("/home/user/Downloads/","NVT_1.0.tar.gz"),
repos=NULL, type="source")
```

2   Standard work flow

2.1   Load data

We need some gene expression data, load the example data provided with the package

```
library("NVT")
```

2.1.1   Load expression data

load example data

```
data(mylen)
data(myexp1)
data(myexp2)
mylist1=c('CT462','CT115','CT045','CT678')
```

2.1.2   Load gene length data

Instead of using a simple flat file, it is also possible to load the gene or exon length data directly from an annotation file in gtf or gff format.

```
mygffpath<-system.file("extdata", "Ctr-D-UW3CX.gff", package = "NVT")
mylen <- NVTloadgff(mygffpath,"gff3","gene","locus_tag")
head(mylen)
```

```
##         Length
## CT001      273
## CT002      303
## CT003     1476
## CT004     1467
## CT005     1092
## CT006      570
```

2.2   Generate NVTdata objects

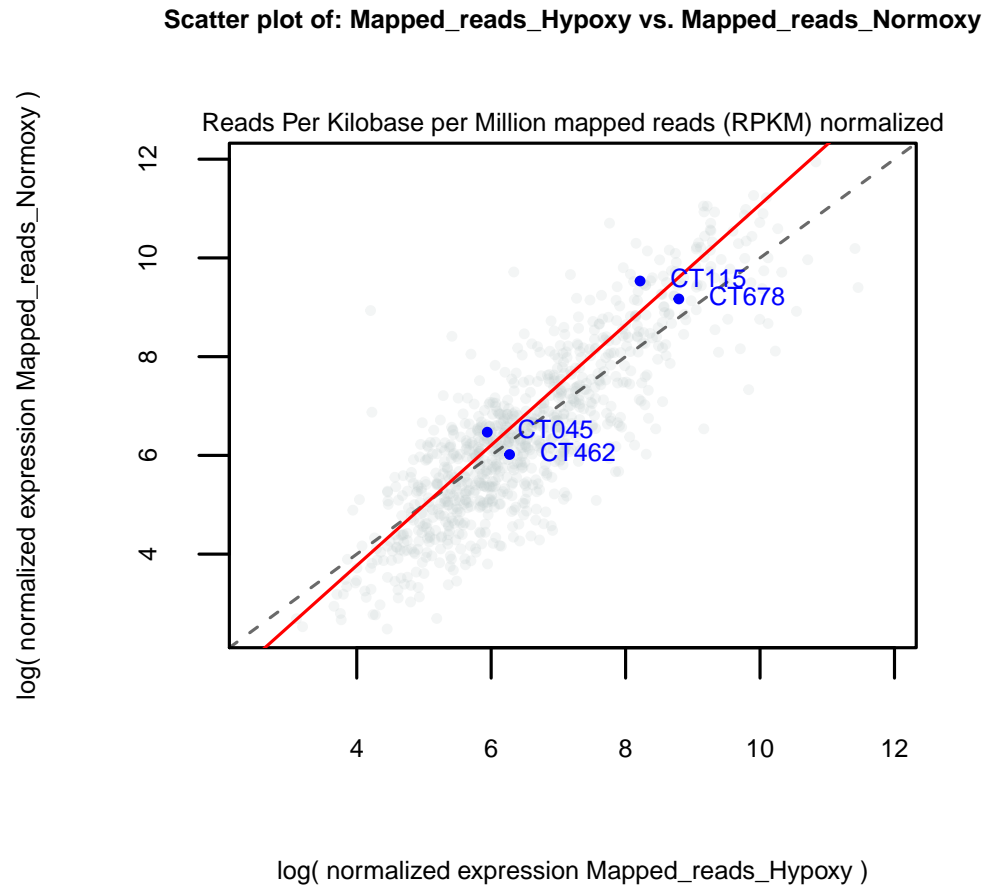**Scatter plot of: Mapped_reads_Hypoxy vs. Mapped_reads_Normoxy**

Figure 1: **Simple XY-Scatter-Plot.** This plot shows the simple NVT plot function result

```
mynvt <- NVTinit(mylist1,myexp1,myexp2,"RPKM",mylen)
```

2.2.1 Normalize the NVTdata

```
mynorm <- NVTnormalize(mynvt)
## [1] "RPKM normalization!"
```

2.3 Visualize expression data

2.3.1 Simple plot
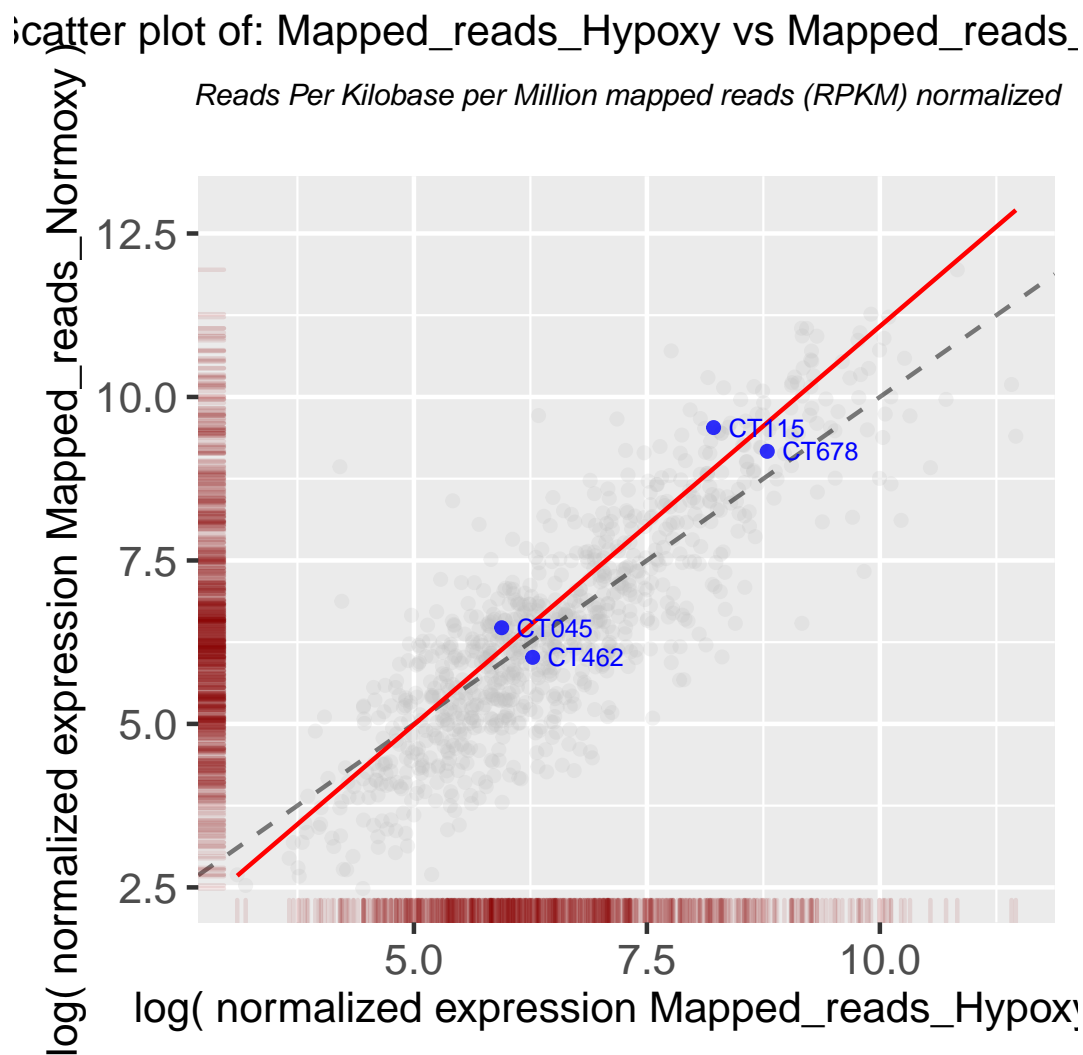
```
NVTplot(mynorm,0.5)
```

Figure 2: **Advanced XY-Scatter-Plot.** This plot shows the advanced NVT plot function result

2.3.2   Advanced plot

```
NVTadvancedplot(mynorm,1,1,2)
```

2.4   Correlation values

2.4.1   Pearson correlation

2.4.2   RMSD and MEA correlation

2.5   Test all methods

3   Acknowledgments

We have benefited in the development of *NVT* from the help and feedback of many individuals,

4   Session Info