Allele-specific ChiPseq analysis

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1) Creation of a union peak list/set with DiffBind:

a) Peak filtering
b) Sample filtering: Some samples have very low peaks.
c) union list https://bioconductor.org/packages/release/bioc/html/DiffBind.html

Currently, peaks are called for each sample individually. Next, you need to determine which peaks are "valid", e.g. a peak that was called in at least 10% of the samples is valid. Note: VALID peaks should first be assessed separately for DEX and VEHICLE conditions and for the analysis the union of these lists should be used.

2) Differential analysis. Usually, in ChIPseq you have an input or IgG control and you would use special tools for the differential analysis. This design is different and differential analysis can be carried out very similar to the corresponding RNAseq data analysis in DEseq or edgeR for treatment condition (veh vs. dex). You can also run differential analysis in DiffBind (which calls DeSeq)

3) Allele-specific binding: generate allele-specific count matrix and perform RasQUAL (R-package)

```
if (!requireNamespace("BiocManager", quietly = TRUE)){
  install.packages("BiocManager")
  BiocManager::install(version = "3.12")
}

if (!require(DiffBind))
  BiocManager::install("DiffBind")
library(DiffBind)

library(GenomicFeatures)

if (!require(ChIPseeker))
  BiocManager::install("ChIPseeker")
library(ChIPseeker)

if (!require(TxDb.Hsapiens.UCSC.hg19.knownGene))
  BiocManager::install("TxDb.Hsapiens.UCSC.hg19.knownGene")
library(TxDb.Hsapiens.UCSC.hg19.knownGene)

if(!require(dplyr))
  install.packages("dplyr")
library(dplyr)
```

1. Read in sampleSheet

```
wd <- "/Users/anastasiia_hry/github/mpip/signe_chip-seq/"
setwd(wd)

result.dir.fn <- paste0(wd, "03_result/")

sample.sheet.fn <- paste0(wd, "01_sample_sheets/chipseq_diffbind_sample_sheet_local.csv")
```

```
samples <- read.csv2(sample.sheet.fn, head = T)[, -11]
```

```
head(samples, 5)
```

```
##          SampleID Tissue Factor Condition Treatment Replicate
## 1 MPIPSYKL_000637     NA   ATAC       low       veh         1
## 2 MPIPSYKL_002418     NA   ATAC       low       veh         1
## 3 MPIPSYKL_003787     NA   ATAC      high       dex         2
## 4 MPIPSYKL_001701     NA   ATAC      high       veh         1
## 5 MPIPSYKL_003231     NA   ATAC      high       veh         1
##
## 1  /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/07_filter_CNV/mpg_L18015_7C_veh_S26.cutadapt.s
## 2 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/07_filter_CNV/mpg_L18015_14D_veh_S33.cutadapt.s
## 3 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/07_filter_CNV/mpg_L18015_15B_dex_S72.cutadapt.s
## 4 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/07_filter_CNV/mpg_L18015_14B_veh_S60.cutadapt.s
## 5 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/07_filter_CNV/mpg_L18015_18D_veh_S62.cutadapt.s
##
## 1  /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/06_MASC2_callpeak/mpg_L18015_7C_veh_S26_peaks
## 2 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/06_MASC2_callpeak/mpg_L18015_14D_veh_S33_peaks
## 3 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/06_MASC2_callpeak/mpg_L18015_15B_dex_S72_peaks
## 4 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/06_MASC2_callpeak/mpg_L18015_14B_veh_S60_peaks
## 5 /Users/anastasiia_hry/Documents/MPIP/SIgne/08_peaks/06_MASC2_callpeak/mpg_L18015_18D_veh_S62_peaks
##   PeakCaller PeakFormat
## 1       macs     narrow
## 2       macs     narrow
## 3       macs     narrow
## 4       macs     narrow
## 5       macs     narrow
```

2. Create and save a DBA object

```
dba.db <- dba(sampleSheet = samples)
```

```
## MPIPSYKL_000637  ATAC low veh 1 macs

## MPIPSYKL_002418  ATAC low veh 1 macs

## MPIPSYKL_003787  ATAC high dex 2 macs

## MPIPSYKL_001701  ATAC high veh 1 macs

## MPIPSYKL_003231  ATAC high veh 1 macs
```

```
## MPIPSYKL_004171  ATAC low dex 2 macs
## MPIPSYKL_002604  ATAC low dex 2 macs
## MPIPSYKL_004054  ATAC low dex 2 macs
## MPIPSYKL_001263  ATAC high veh 1 macs
## MPIPSYKL_001786  ATAC high veh 1 macs
## MPIPSYKL_001946  ATAC high veh 1 macs
## MPIPSYKL_001113  ATAC high dex 2 macs
## MPIPSYKL_000972  ATAC low veh 1 macs
## MPIPSYKL_004100  ATAC low dex 2 macs
## MPIPSYKL_003331  ATAC low veh 1 macs
## MPIPSYKL_003520  ATAC high veh 1 macs
## MPIPSYKL_000988  ATAC high veh 1 macs
## MPIPSYKL_004171  ATAC low veh 1 macs
## MPIPSYKL_003331  ATAC low dex 2 macs
## MPIPSYKL_001766  ATAC low veh 1 macs
## MPIPSYKL_003520  ATAC high dex 2 macs
## MPIPSYKL_004215  ATAC high dex 2 macs
## MPIPSYKL_001946  ATAC high dex 2 macs
## MPIPSYKL_003353  ATAC low veh 1 macs
## MPIPSYKL_002319  ATAC low veh 1 macs
## MPIPSYKL_001109  ATAC high veh 1 macs
## MPIPSYKL_004251  ATAC high veh 1 macs
## MPIPSYKL_000708  ATAC low veh 1 macs
## MPIPSYKL_004251  ATAC high dex 2 macs
```

```
## MPIPSYKL_002802  ATAC low dex 2 macs
## MPIPSYKL_002095  ATAC low veh 1 macs
## MPIPSYKL_003162  ATAC low dex 2 macs
## MPIPSYKL_002468  ATAC high veh 1 macs
## MPIPSYKL_002658  ATAC low dex 2 macs
## MPIPSYKL_003472  ATAC high veh 1 macs
## MPIPSYKL_004054  ATAC low veh 1 macs
## MPIPSYKL_002613  ATAC high dex 2 macs
## MPIPSYKL_003231  ATAC high dex 2 macs
## MPIPSYKL_002533  ATAC high dex 2 macs
## MPIPSYKL_002802  ATAC low veh 1 macs
## MPIPSYKL_003934  ATAC high veh 1 macs
## MPIPSYKL_002418  ATAC low dex 2 macs
## MPIPSYKL_001109  ATAC high dex 2 macs
## MPIPSYKL_001786  ATAC high dex 2 macs
## MPIPSYKL_003934  ATAC high dex 2 macs
## MPIPSYKL_002383  ATAC low veh 1 macs
## MPIPSYKL_004215  ATAC high veh 1 macs
## MPIPSYKL_004246  ATAC high dex 2 macs
## MPIPSYKL_001113  ATAC high veh 1 macs
## MPIPSYKL_001547  ATAC high dex 2 macs
## MPIPSYKL_001667  ATAC high veh 1 macs
## MPIPSYKL_000637  ATAC low dex 2 macs
## MPIPSYKL_001386  ATAC high dex 2 macs
```

```
## MPIPSYKL_002468  ATAC high dex 2 macs
## MPIPSYKL_002088  ATAC low dex 2 macs
## MPIPSYKL_003013  ATAC high veh 1 macs
## MPIPSYKL_000972  ATAC low dex 2 macs
## MPIPSYKL_001048  ATAC high veh 1 macs
## MPIPSYKL_001389  ATAC low veh 1 macs
## MPIPSYKL_003785  ATAC high dex 2 macs
## MPIPSYKL_003155  ATAC low veh 1 macs
## MPIPSYKL_001886  ATAC low veh 1 macs
## MPIPSYKL_003787  ATAC high veh 1 macs
## MPIPSYKL_004246  ATAC high veh 1 macs
## MPIPSYKL_002852  ATAC low veh 1 macs
## MPIPSYKL_002613  ATAC high veh 1 macs
## MPIPSYKL_001389  ATAC low dex 2 macs
## MPIPSYKL_003155  ATAC low dex 2 macs
## MPIPSYKL_000708  ATAC low dex 2 macs
## MPIPSYKL_003911  ATAC low veh 1 macs
## MPIPSYKL_001886  ATAC low dex 2 macs
## MPIPSYKL_003013  ATAC high dex 2 macs
## MPIPSYKL_002604  ATAC low veh 1 macs
## MPIPSYKL_002533  ATAC high veh 1 macs
## MPIPSYKL_001547  ATAC high veh 1 macs
## MPIPSYKL_000988  ATAC high dex 2 macs
## MPIPSYKL_004100  ATAC low veh 1 macs
```

```
## MPIPSYKL_003472  ATAC high dex 2 macs

## MPIPSYKL_001386  ATAC high veh 1 macs

## MPIPSYKL_003911  ATAC low dex 2 macs

## MPIPSYKL_003162  ATAC low veh 1 macs

## MPIPSYKL_001701  ATAC high dex 2 macs

## MPIPSYKL_002383  ATAC low dex 2 macs

## MPIPSYKL_002658  ATAC low veh 1 macs

## MPIPSYKL_001766  ATAC low dex 2 macs

## MPIPSYKL_002852  ATAC low dex 2 macs

## MPIPSYKL_001263  ATAC high dex 2 macs

## MPIPSYKL_004198  ATAC low veh 1 macs

## MPIPSYKL_003353  ATAC low dex 2 macs

## MPIPSYKL_001667  ATAC high dex 2 macs

## MPIPSYKL_002319  ATAC low dex 2 macs

## MPIPSYKL_003785  ATAC high veh 1 macs

## MPIPSYKL_001048  ATAC high dex 2 macs

## MPIPSYKL_002095  ATAC low dex 2 macs

## MPIPSYKL_002088  ATAC low veh 1 macs
```

```
# dba.save(dba.db, file = "Signe_ChIPseq", dir = result.dir.fn)
```

```
# load(paste0(result.dir.fn, "dba_Signe_ChIPseq"))
dba.db
```

```
## 95 Samples, 24124 sites in matrix (44967 total):
##                 ID Factor Condition Treatment Replicate Intervals
## 1  MPIPSYKL_000637   ATAC       low       veh         1       460
## 2  MPIPSYKL_002418   ATAC       low       veh         1       354
## 3  MPIPSYKL_003787   ATAC      high       dex         2      1955
## 4  MPIPSYKL_001701   ATAC      high       veh         1      9725
## 5  MPIPSYKL_003231   ATAC      high       veh         1       236
## 6  MPIPSYKL_004171   ATAC       low       dex         2      2699
```

```
## 7  MPIPSYKL_002604   ATAC       low       dex         2      1290
## 8  MPIPSYKL_004054   ATAC       low       dex         2      2302
## 9  MPIPSYKL_001263   ATAC      high       veh         1       413
## 10 MPIPSYKL_001786   ATAC      high       veh         1       977
## 11 MPIPSYKL_001946   ATAC      high       veh         1       771
## 12 MPIPSYKL_001113   ATAC      high       dex         2       204
## 13 MPIPSYKL_000972   ATAC       low       veh         1       581
## 14 MPIPSYKL_004100   ATAC       low       dex         2     19759
## 15 MPIPSYKL_003331   ATAC       low       veh         1      3347
## 16 MPIPSYKL_003520   ATAC      high       veh         1       192
## 17 MPIPSYKL_000988   ATAC      high       veh         1      1493
## 18 MPIPSYKL_004171   ATAC       low       veh         1       346
## 19 MPIPSYKL_003331   ATAC       low       dex         2      1706
## 20 MPIPSYKL_001766   ATAC       low       veh         1       136
## 21 MPIPSYKL_003520   ATAC      high       dex         2      2950
## 22 MPIPSYKL_004215   ATAC      high       dex         2      2356
## 23 MPIPSYKL_001946   ATAC      high       dex         2     17256
## 24 MPIPSYKL_003353   ATAC       low       veh         1      3097
## 25 MPIPSYKL_002319   ATAC       low       veh         1       496
## 26 MPIPSYKL_001109   ATAC      high       veh         1      2130
## 27 MPIPSYKL_004251   ATAC      high       veh         1       410
## 28 MPIPSYKL_000708   ATAC       low       veh         1       760
## 29 MPIPSYKL_004251   ATAC      high       dex         2       563
## 30 MPIPSYKL_002802   ATAC       low       dex         2      1617
## 31 MPIPSYKL_002095   ATAC       low       veh         1       237
## 32 MPIPSYKL_003162   ATAC       low       dex         2       787
## 33 MPIPSYKL_002468   ATAC      high       veh         1       247
## 34 MPIPSYKL_002658   ATAC       low       dex         2      4977
## 35 MPIPSYKL_003472   ATAC      high       veh         1       195
## 36 MPIPSYKL_004054   ATAC       low       veh         1      3423
## 37 MPIPSYKL_002613   ATAC      high       dex         2       875
## 38 MPIPSYKL_003231   ATAC      high       dex         2      2073
## 39 MPIPSYKL_002533   ATAC      high       dex         2      1120
## 40 MPIPSYKL_002802   ATAC       low       veh         1       838
## 41 MPIPSYKL_003934   ATAC      high       veh         1       259
## 42 MPIPSYKL_002418   ATAC       low       dex         2       708
## 43 MPIPSYKL_001109   ATAC      high       dex         2       620
## 44 MPIPSYKL_001786   ATAC      high       dex         2       858
## 45 MPIPSYKL_003934   ATAC      high       dex         2      7158
## 46 MPIPSYKL_002383   ATAC       low       veh         1       492
## 47 MPIPSYKL_004215   ATAC      high       veh         1       400
## 48 MPIPSYKL_004246   ATAC      high       dex         2      1145
## 49 MPIPSYKL_001113   ATAC      high       veh         1       242
## 50 MPIPSYKL_001547   ATAC      high       dex         2       253
## 51 MPIPSYKL_001667   ATAC      high       veh         1       190
## 52 MPIPSYKL_000637   ATAC       low       dex         2      1141
## 53 MPIPSYKL_001386   ATAC      high       dex         2       785
## 54 MPIPSYKL_002468   ATAC      high       dex         2      1601
## 55 MPIPSYKL_002088   ATAC       low       dex         2      4266
## 56 MPIPSYKL_003013   ATAC      high       veh         1      2791
## 57 MPIPSYKL_000972   ATAC       low       dex         2      3099
## 58 MPIPSYKL_001048   ATAC      high       veh         1      3022
## 59 MPIPSYKL_001389   ATAC       low       veh         1       635
## 60 MPIPSYKL_003785   ATAC      high       dex         2      9905
```

```
## 61 MPIPSYKL_003155   ATAC       low       veh         1       922
## 62 MPIPSYKL_001886   ATAC       low       veh         1      2438
## 63 MPIPSYKL_003787   ATAC      high       veh         1       178
## 64 MPIPSYKL_004246   ATAC      high       veh         1       579
## 65 MPIPSYKL_002852   ATAC       low       veh         1       365
## 66 MPIPSYKL_002613   ATAC      high       veh         1      4060
## 67 MPIPSYKL_001389   ATAC       low       dex         2      2622
## 68 MPIPSYKL_003155   ATAC       low       dex         2      1290
## 69 MPIPSYKL_000708   ATAC       low       dex         2      1956
## 70 MPIPSYKL_003911   ATAC       low       veh         1       246
## 71 MPIPSYKL_001886   ATAC       low       dex         2      5353
## 72 MPIPSYKL_003013   ATAC      high       dex         2      1467
## 73 MPIPSYKL_002604   ATAC       low       veh         1       205
## 74 MPIPSYKL_002533   ATAC      high       veh         1       302
## 75 MPIPSYKL_001547   ATAC      high       veh         1       142
## 76 MPIPSYKL_000988   ATAC      high       dex         2      4918
## 77 MPIPSYKL_004100   ATAC       low       veh         1       598
## 78 MPIPSYKL_003472   ATAC      high       dex         2       375
## 79 MPIPSYKL_001386   ATAC      high       veh         1       235
## 80 MPIPSYKL_003911   ATAC       low       dex         2       470
## 81 MPIPSYKL_003162   ATAC       low       veh         1       219
## 82 MPIPSYKL_001701   ATAC      high       dex         2      3480
## 83 MPIPSYKL_002383   ATAC       low       dex         2       836
## 84 MPIPSYKL_002658   ATAC       low       veh         1       181
## 85 MPIPSYKL_001766   ATAC       low       dex         2     11025
## 86 MPIPSYKL_002852   ATAC       low       dex         2      8121
## 87 MPIPSYKL_001263   ATAC      high       dex         2      2089
## 88 MPIPSYKL_004198   ATAC       low       veh         1       181
## 89 MPIPSYKL_003353   ATAC       low       dex         2     11524
## 90 MPIPSYKL_001667   ATAC      high       dex         2      5503
## 91 MPIPSYKL_002319   ATAC       low       dex         2       831
## 92 MPIPSYKL_003785   ATAC      high       veh         1      6017
## 93 MPIPSYKL_001048   ATAC      high       dex         2      2068
## 94 MPIPSYKL_002095   ATAC       low       dex         2     19071
## 95 MPIPSYKL_002088   ATAC       low       veh         1      3154
```

This shows how many peaks are in each peakset, as well as total number of unique peaks after merging overlapping ones (44'967) and the default binding matrix of 95 samples by the 24'124 sites that overlap in at least two of the samples.

3. Peaksets overlap rate

To determine which peaks are "valid", we perform overlap peaksets analysis for each group (dex and veh) separately.

3.1. Dex group

```
dba.overlap(dba.db, dba.db$masks$dex, mode=DBA_OLAP_RATE)
```

```
##  [1] 37757 21482 16679 13616 11128  9285  7674  6495  5523  4707  4062  3458
## [13]  3071  2731  2428  2180  1986  1832  1671  1519  1401  1307  1238  1156
## [25]  1085  1016   932   890   838   774   719   683   632   577   541   500
## [37]   459   419   385   343   306   278   242   216   184   139    93
```

47 consensus peaksets were identified for dex group, 37'757 of which is the total number of unique sites, 21'482 - the number of unique sites appearing in at least two peaksets, 16'679 - the number of sites overlapping in at least three peaksets, etc.

```
dba.overlap(dba.db, dba.db$masks$dex & dba.db$masks$high, mode=DBA_OLAP_RATE)
```

```
##  [1] 24355 11496  7477  5285  3870  2981  2350  1946  1644  1408  1241  1112
## [13]   973   845   747   665   582   496   407   328   263   206   150    99
```

24 consensus peaksets were identified for dex group, 24'355 of which is the total number of unique sites, 11'496 - the number of unique sites appearing in at least two peaksets, 7'477 - the number of sites overlapping in at least three peaksets, etc.

3.2. Veh group

```
dba.overlap(dba.db, dba.db$masks$veh, mode=DBA_OLAP_RATE)
```

```
##  [1] 21486  8772  5760  4118  3039  2287  1716  1346  1047   832   703   576
## [13]   478   426   386   336   307   281   251   224   208   188   180   175
## [25]   163   153   143   139   131   127   124   120   114   111   107   101
## [37]    98    97    93    90    87    80    77    75    72    67    62    53
```

48 consensus peaksets were identified for dex group, 21'486 of which is the total number of unique sites, 8'772 - the number of unique sites appearing in at least two peaksets, 5'760 - the number of sites overlapping in at least three peaksets, etc.

```
dba.overlap(dba.db, dba.db$masks$veh & dba.db$masks$high, mode=DBA_OLAP_RATE)
```

```
##  [1] 17310  6567  3583  2090  1219   751   531   397   317   262   212   178
## [13]   157   141   134   121   111   104    96    87    82    77    68    55
```

4. Create separate consensus peaksets for each of the two treatments (veh and dex), then take the union of these two peaksets as the overall consensus

Requiring that consensus peaks overlap in at least 20% of the samples in each group results in 3039 sites for the veh group and 11'128 sites for the dex group:

```
dba.consensus <- dba.peakset(dba.db, consensus = c(DBA_TREATMENT), minOverlap = 0.2)
```

```
## Add consensus: veh
```

```
## Add consensus: dex
```

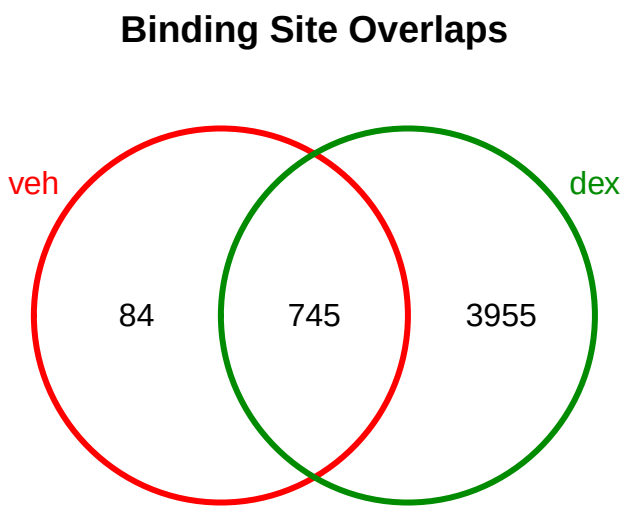
```
dba.consensus
```

```
## 97 Samples, 24124 sites in matrix (44967 total):
##                 ID Factor Condition Treatment Replicate Intervals
## 1  MPIPSYKL_000637   ATAC       low       veh         1       460
## 2  MPIPSYKL_002418   ATAC       low       veh         1       354
## 3  MPIPSYKL_003787   ATAC      high       dex         2      1955
```

```
## 4  MPIPSYKL_001701   ATAC      high       veh         1      9725
## 5  MPIPSYKL_003231   ATAC      high       veh         1       236
## 6  MPIPSYKL_004171   ATAC       low       dex         2      2699
## 7  MPIPSYKL_002604   ATAC       low       dex         2      1290
## 8  MPIPSYKL_004054   ATAC       low       dex         2      2302
## 9  MPIPSYKL_001263   ATAC      high       veh         1       413
## 10 MPIPSYKL_001786   ATAC      high       veh         1       977
## 11 MPIPSYKL_001946   ATAC      high       veh         1       771
## 12 MPIPSYKL_001113   ATAC      high       dex         2       204
## 13 MPIPSYKL_000972   ATAC       low       veh         1       581
## 14 MPIPSYKL_004100   ATAC       low       dex         2     19759
## 15 MPIPSYKL_003331   ATAC       low       veh         1      3347
## 16 MPIPSYKL_003520   ATAC      high       veh         1       192
## 17 MPIPSYKL_000988   ATAC      high       veh         1      1493
## 18 MPIPSYKL_004171   ATAC       low       veh         1       346
## 19 MPIPSYKL_003331   ATAC       low       dex         2      1706
## 20 MPIPSYKL_001766   ATAC       low       veh         1       136
## 21 MPIPSYKL_003520   ATAC      high       dex         2      2950
## 22 MPIPSYKL_004215   ATAC      high       dex         2      2356
## 23 MPIPSYKL_001946   ATAC      high       dex         2     17256
## 24 MPIPSYKL_003353   ATAC       low       veh         1      3097
## 25 MPIPSYKL_002319   ATAC       low       veh         1       496
## 26 MPIPSYKL_001109   ATAC      high       veh         1      2130
## 27 MPIPSYKL_004251   ATAC      high       veh         1       410
## 28 MPIPSYKL_000708   ATAC       low       veh         1       760
## 29 MPIPSYKL_004251   ATAC      high       dex         2       563
## 30 MPIPSYKL_002802   ATAC       low       dex         2      1617
## 31 MPIPSYKL_002095   ATAC       low       veh         1       237
## 32 MPIPSYKL_003162   ATAC       low       dex         2       787
## 33 MPIPSYKL_002468   ATAC      high       veh         1       247
## 34 MPIPSYKL_002658   ATAC       low       dex         2      4977
## 35 MPIPSYKL_003472   ATAC      high       veh         1       195
## 36 MPIPSYKL_004054   ATAC       low       veh         1      3423
## 37 MPIPSYKL_002613   ATAC      high       dex         2       875
## 38 MPIPSYKL_003231   ATAC      high       dex         2      2073
## 39 MPIPSYKL_002533   ATAC      high       dex         2      1120
## 40 MPIPSYKL_002802   ATAC       low       veh         1       838
## 41 MPIPSYKL_003934   ATAC      high       veh         1       259
## 42 MPIPSYKL_002418   ATAC       low       dex         2       708
## 43 MPIPSYKL_001109   ATAC      high       dex         2       620
## 44 MPIPSYKL_001786   ATAC      high       dex         2       858
## 45 MPIPSYKL_003934   ATAC      high       dex         2      7158
## 46 MPIPSYKL_002383   ATAC       low       veh         1       492
## 47 MPIPSYKL_004215   ATAC      high       veh         1       400
## 48 MPIPSYKL_004246   ATAC      high       dex         2      1145
## 49 MPIPSYKL_001113   ATAC      high       veh         1       242
## 50 MPIPSYKL_001547   ATAC      high       dex         2       253
## 51 MPIPSYKL_001667   ATAC      high       veh         1       190
## 52 MPIPSYKL_000637   ATAC       low       dex         2      1141
## 53 MPIPSYKL_001386   ATAC      high       dex         2       785
## 54 MPIPSYKL_002468   ATAC      high       dex         2      1601
## 55 MPIPSYKL_002088   ATAC       low       dex         2      4266
## 56 MPIPSYKL_003013   ATAC      high       veh         1      2791
## 57 MPIPSYKL_000972   ATAC       low       dex         2      3099
```

```
## 58 MPIPSYKL_001048   ATAC      high       veh         1      3022
## 59 MPIPSYKL_001389   ATAC       low       veh         1       635
## 60 MPIPSYKL_003785   ATAC      high       dex         2      9905
## 61 MPIPSYKL_003155   ATAC       low       veh         1       922
## 62 MPIPSYKL_001886   ATAC       low       veh         1      2438
## 63 MPIPSYKL_003787   ATAC      high       veh         1       178
## 64 MPIPSYKL_004246   ATAC      high       veh         1       579
## 65 MPIPSYKL_002852   ATAC       low       veh         1       365
## 66 MPIPSYKL_002613   ATAC      high       veh         1      4060
## 67 MPIPSYKL_001389   ATAC       low       dex         2      2622
## 68 MPIPSYKL_003155   ATAC       low       dex         2      1290
## 69 MPIPSYKL_000708   ATAC       low       dex         2      1956
## 70 MPIPSYKL_003911   ATAC       low       veh         1       246
## 71 MPIPSYKL_001886   ATAC       low       dex         2      5353
## 72 MPIPSYKL_003013   ATAC      high       dex         2      1467
## 73 MPIPSYKL_002604   ATAC       low       veh         1       205
## 74 MPIPSYKL_002533   ATAC      high       veh         1       302
## 75 MPIPSYKL_001547   ATAC      high       veh         1       142
## 76 MPIPSYKL_000988   ATAC      high       dex         2      4918
## 77 MPIPSYKL_004100   ATAC       low       veh         1       598
## 78 MPIPSYKL_003472   ATAC      high       dex         2       375
## 79 MPIPSYKL_001386   ATAC      high       veh         1       235
## 80 MPIPSYKL_003911   ATAC       low       dex         2       470
## 81 MPIPSYKL_003162   ATAC       low       veh         1       219
## 82 MPIPSYKL_001701   ATAC      high       dex         2      3480
## 83 MPIPSYKL_002383   ATAC       low       dex         2       836
## 84 MPIPSYKL_002658   ATAC       low       veh         1       181
## 85 MPIPSYKL_001766   ATAC       low       dex         2     11025
## 86 MPIPSYKL_002852   ATAC       low       dex         2      8121
## 87 MPIPSYKL_001263   ATAC      high       dex         2      2089
## 88 MPIPSYKL_004198   ATAC       low       veh         1       181
## 89 MPIPSYKL_003353   ATAC       low       dex         2     11524
## 90 MPIPSYKL_001667   ATAC      high       dex         2      5503
## 91 MPIPSYKL_002319   ATAC       low       dex         2       831
## 92 MPIPSYKL_003785   ATAC      high       veh         1      6017
## 93 MPIPSYKL_001048   ATAC      high       dex         2      2068
## 94 MPIPSYKL_002095   ATAC       low       dex         2     19071
## 95 MPIPSYKL_002088   ATAC       low       veh         1      3154
## 96             veh   ATAC  low-high       veh         1       832
## 97             dex   ATAC  high-low       dex         2      4707
```

```
dba.plotVenn(dba.consensus, dba.consensus$masks$Consensus)
```



5. Merge peakset list

When forming the global binding matrix consensus peaksets, DiffBind first identifies all unique peaks amongst the relevant peaksets. As part of this process, it merges overlapping peaks, replacing them with a single peak representing the narrowest region that covers all peaks that overlap by at least one base.

Add a consensus peakset (derived from overlapping peaks in peaksets already present) :

```
dba.consensus.merge <- dba.peakset(dba.consensus, peaks = dba.consensus$masks$Consensus, minOverlap = 1)
dba.consensus.merge
```

```
## 98 Samples, 24124 sites in matrix (44967 total):
##                 ID Factor         Condition Treatment Replicate Intervals
## 1  MPIPSYKL_000637   ATAC               low       veh         1       460
## 2  MPIPSYKL_002418   ATAC               low       veh         1       354
## 3  MPIPSYKL_003787   ATAC              high       dex         2      1955
## 4  MPIPSYKL_001701   ATAC              high       veh         1      9725
## 5  MPIPSYKL_003231   ATAC              high       veh         1       236
## 6  MPIPSYKL_004171   ATAC               low       dex         2      2699
## 7  MPIPSYKL_002604   ATAC               low       dex         2      1290
## 8  MPIPSYKL_004054   ATAC               low       dex         2      2302
## 9  MPIPSYKL_001263   ATAC              high       veh         1       413
## 10 MPIPSYKL_001786   ATAC              high       veh         1       977
## 11 MPIPSYKL_001946   ATAC              high       veh         1       771
## 12 MPIPSYKL_001113   ATAC              high       dex         2       204
```

```
## 13 MPIPSYKL_000972   ATAC              low       veh         1       581
## 14 MPIPSYKL_004100   ATAC              low       dex         2     19759
## 15 MPIPSYKL_003331   ATAC              low       veh         1      3347
## 16 MPIPSYKL_003520   ATAC             high       veh         1       192
## 17 MPIPSYKL_000988   ATAC             high       veh         1      1493
## 18 MPIPSYKL_004171   ATAC              low       veh         1       346
## 19 MPIPSYKL_003331   ATAC              low       dex         2      1706
## 20 MPIPSYKL_001766   ATAC              low       veh         1       136
## 21 MPIPSYKL_003520   ATAC             high       dex         2      2950
## 22 MPIPSYKL_004215   ATAC             high       dex         2      2356
## 23 MPIPSYKL_001946   ATAC             high       dex         2     17256
## 24 MPIPSYKL_003353   ATAC              low       veh         1      3097
## 25 MPIPSYKL_002319   ATAC              low       veh         1       496
## 26 MPIPSYKL_001109   ATAC             high       veh         1      2130
## 27 MPIPSYKL_004251   ATAC             high       veh         1       410
## 28 MPIPSYKL_000708   ATAC              low       veh         1       760
## 29 MPIPSYKL_004251   ATAC             high       dex         2       563
## 30 MPIPSYKL_002802   ATAC              low       dex         2      1617
## 31 MPIPSYKL_002095   ATAC              low       veh         1       237
## 32 MPIPSYKL_003162   ATAC              low       dex         2       787
## 33 MPIPSYKL_002468   ATAC             high       veh         1       247
## 34 MPIPSYKL_002658   ATAC              low       dex         2      4977
## 35 MPIPSYKL_003472   ATAC             high       veh         1       195
## 36 MPIPSYKL_004054   ATAC              low       veh         1      3423
## 37 MPIPSYKL_002613   ATAC             high       dex         2       875
## 38 MPIPSYKL_003231   ATAC             high       dex         2      2073
## 39 MPIPSYKL_002533   ATAC             high       dex         2      1120
## 40 MPIPSYKL_002802   ATAC              low       veh         1       838
## 41 MPIPSYKL_003934   ATAC             high       veh         1       259
## 42 MPIPSYKL_002418   ATAC              low       dex         2       708
## 43 MPIPSYKL_001109   ATAC             high       dex         2       620
## 44 MPIPSYKL_001786   ATAC             high       dex         2       858
## 45 MPIPSYKL_003934   ATAC             high       dex         2      7158
## 46 MPIPSYKL_002383   ATAC              low       veh         1       492
## 47 MPIPSYKL_004215   ATAC             high       veh         1       400
## 48 MPIPSYKL_004246   ATAC             high       dex         2      1145
## 49 MPIPSYKL_001113   ATAC             high       veh         1       242
## 50 MPIPSYKL_001547   ATAC             high       dex         2       253
## 51 MPIPSYKL_001667   ATAC             high       veh         1       190
## 52 MPIPSYKL_000637   ATAC              low       dex         2      1141
## 53 MPIPSYKL_001386   ATAC             high       dex         2       785
## 54 MPIPSYKL_002468   ATAC             high       dex         2      1601
## 55 MPIPSYKL_002088   ATAC              low       dex         2      4266
## 56 MPIPSYKL_003013   ATAC             high       veh         1      2791
## 57 MPIPSYKL_000972   ATAC              low       dex         2      3099
## 58 MPIPSYKL_001048   ATAC             high       veh         1      3022
## 59 MPIPSYKL_001389   ATAC              low       veh         1       635
## 60 MPIPSYKL_003785   ATAC             high       dex         2      9905
## 61 MPIPSYKL_003155   ATAC              low       veh         1       922
## 62 MPIPSYKL_001886   ATAC              low       veh         1      2438
## 63 MPIPSYKL_003787   ATAC             high       veh         1       178
## 64 MPIPSYKL_004246   ATAC             high       veh         1       579
## 65 MPIPSYKL_002852   ATAC              low       veh         1       365
## 66 MPIPSYKL_002613   ATAC             high       veh         1      4060
```

```
## 67 MPIPSYKL_001389   ATAC              low       dex         2      2622
## 68 MPIPSYKL_003155   ATAC              low       dex         2      1290
## 69 MPIPSYKL_000708   ATAC              low       dex         2      1956
## 70 MPIPSYKL_003911   ATAC              low       veh         1       246
## 71 MPIPSYKL_001886   ATAC              low       dex         2      5353
## 72 MPIPSYKL_003013   ATAC             high       dex         2      1467
## 73 MPIPSYKL_002604   ATAC              low       veh         1       205
## 74 MPIPSYKL_002533   ATAC             high       veh         1       302
## 75 MPIPSYKL_001547   ATAC             high       veh         1       142
## 76 MPIPSYKL_000988   ATAC             high       dex         2      4918
## 77 MPIPSYKL_004100   ATAC              low       veh         1       598
## 78 MPIPSYKL_003472   ATAC             high       dex         2       375
## 79 MPIPSYKL_001386   ATAC             high       veh         1       235
## 80 MPIPSYKL_003911   ATAC              low       dex         2       470
## 81 MPIPSYKL_003162   ATAC              low       veh         1       219
## 82 MPIPSYKL_001701   ATAC             high       dex         2      3480
## 83 MPIPSYKL_002383   ATAC              low       dex         2       836
## 84 MPIPSYKL_002658   ATAC              low       veh         1       181
## 85 MPIPSYKL_001766   ATAC              low       dex         2     11025
## 86 MPIPSYKL_002852   ATAC              low       dex         2      8121
## 87 MPIPSYKL_001263   ATAC             high       dex         2      2089
## 88 MPIPSYKL_004198   ATAC              low       veh         1       181
## 89 MPIPSYKL_003353   ATAC              low       dex         2     11524
## 90 MPIPSYKL_001667   ATAC             high       dex         2      5503
## 91 MPIPSYKL_002319   ATAC              low       dex         2       831
## 92 MPIPSYKL_003785   ATAC             high       veh         1      6017
## 93 MPIPSYKL_001048   ATAC             high       dex         2      2068
## 94 MPIPSYKL_002095   ATAC              low       dex         2     19071
## 95 MPIPSYKL_002088   ATAC              low       veh         1      3154
## 96             veh   ATAC         low-high       veh         1       832
## 97             dex   ATAC         high-low       dex         2      4707
## 98         veh-dex   ATAC low-high-high-low   veh-dex       1-2      4784
```

Save union peakset :

```
n             <- length(dba.consensus.merge$peaks)
union.peakset <- dba.consensus.merge$peaks[[n]][,1:3]

write.table(union.peakset, file = paste0(result.dir.fn, "union_peakset.tsv"), sep = "\t", row.names = F
```

6. Generate a binding affinity matrix based on read count scores :

```
dba.count.db <- dba.count(dba.db, peaks = union.peakset, bRemoveDuplicates = F, score = DBA_SCORE_READS
save(dba.count.db, file = paste0(result.dir.fn, "dba_count.Rda"))

dba.count.norm.db <- dba.count(dba.db, peaks = union.peakset, bRemoveDuplicates = F)
```

7. Establishing contrast

Next we need to let DiffBind know how we want to group our samples. In our case we will group based on treatment. We also have to set the minMembers parameter to 47 since we have 47 samples in dex and 48 in veh group.

```r
# load(paste0(result.dir.fn, "dba_count.Rda"))
dba.contrast <- dba.contrast(dba.count.db, categories = c(DBA_CONDITION, DBA_TREATMENT), minMembers = 4
```

```
## Computing results names...
```

```r
dba.contrast
```

```
## 95 Samples, 4784 sites in matrix:
##                 ID Factor Condition Treatment Replicate     Reads FRiP
## 1  MPIPSYKL_000637   ATAC       low       veh         1   48646.5 0.48
## 2  MPIPSYKL_002418   ATAC       low       veh         1   39876.0 0.41
## 3  MPIPSYKL_003787   ATAC      high       dex         2  122369.5 0.60
## 4  MPIPSYKL_001701   ATAC      high       veh         1  252860.5 0.28
## 5  MPIPSYKL_003231   ATAC      high       veh         1   32347.0 0.38
## 6  MPIPSYKL_004171   ATAC       low       dex         2  173266.5 0.61
## 7  MPIPSYKL_002604   ATAC       low       dex         2   73592.0 0.63
## 8  MPIPSYKL_004054   ATAC       low       dex         2  133546.5 0.65
## 9  MPIPSYKL_001263   ATAC      high       veh         1   25906.5 0.46
## 10 MPIPSYKL_001786   ATAC      high       veh         1   38769.0 0.35
## 11 MPIPSYKL_001946   ATAC      high       veh         1   44678.0 0.41
## 12 MPIPSYKL_001113   ATAC      high       dex         2   26133.0 0.45
## 13 MPIPSYKL_000972   ATAC       low       veh         1   44139.0 0.42
## 14 MPIPSYKL_004100   ATAC       low       dex         2 1093528.0 0.38
## 15 MPIPSYKL_003331   ATAC       low       veh         1  136884.0 0.33
## 16 MPIPSYKL_003520   ATAC      high       veh         1   34792.5 0.37
## 17 MPIPSYKL_000988   ATAC      high       veh         1   97502.5 0.35
## 18 MPIPSYKL_004171   ATAC       low       veh         1   59254.5 0.34
## 19 MPIPSYKL_003331   ATAC       low       dex         2   80213.0 0.70
## 20 MPIPSYKL_001766   ATAC       low       veh         1   29695.5 0.35
## 21 MPIPSYKL_003520   ATAC      high       dex         2  137986.5 0.72
## 22 MPIPSYKL_004215   ATAC      high       dex         2  120145.0 0.63
## 23 MPIPSYKL_001946   ATAC      high       dex         2 1128909.5 0.51
## 24 MPIPSYKL_003353   ATAC       low       veh         1  136983.5 0.34
## 25 MPIPSYKL_002319   ATAC       low       veh         1   50702.5 0.51
## 26 MPIPSYKL_001109   ATAC      high       veh         1   89583.0 0.32
## 27 MPIPSYKL_004251   ATAC      high       veh         1   35730.5 0.42
## 28 MPIPSYKL_000708   ATAC       low       veh         1   33399.5 0.39
## 29 MPIPSYKL_004251   ATAC      high       dex         2   44901.0 0.41
## 30 MPIPSYKL_002802   ATAC       low       dex         2   79331.0 0.60
## 31 MPIPSYKL_002095   ATAC       low       veh         1   32884.0 0.44
## 32 MPIPSYKL_003162   ATAC       low       dex         2   41535.5 0.51
## 33 MPIPSYKL_002468   ATAC      high       veh         1   38420.0 0.42
## 34 MPIPSYKL_002658   ATAC       low       dex         2  315901.5 0.67
## 35 MPIPSYKL_003472   ATAC      high       veh         1   26032.0 0.47
## 36 MPIPSYKL_004054   ATAC       low       veh         1  174999.5 0.32
## 37 MPIPSYKL_002613   ATAC      high       dex         2   80250.5 0.49
## 38 MPIPSYKL_003231   ATAC      high       dex         2   88498.0 0.65
## 39 MPIPSYKL_002533   ATAC      high       dex         2  113871.0 0.45
## 40 MPIPSYKL_002802   ATAC       low       veh         1   52387.5 0.35
## 41 MPIPSYKL_003934   ATAC      high       veh         1   32213.5 0.39
## 42 MPIPSYKL_002418   ATAC       low       dex         2   56650.5 0.53
## 43 MPIPSYKL_001109   ATAC      high       dex         2   45694.0 0.56
## 44 MPIPSYKL_001786   ATAC      high       dex         2   65412.0 0.55
```

```
## 45 MPIPSYKL_003934   ATAC      high       dex         2  357234.0 0.60
## 46 MPIPSYKL_002383   ATAC       low       veh         1   38184.5 0.35
## 47 MPIPSYKL_004215   ATAC      high       veh         1   31424.0 0.48
## 48 MPIPSYKL_004246   ATAC      high       dex         2   86453.5 0.62
## 49 MPIPSYKL_001113   ATAC      high       veh         1   36781.5 0.35
## 50 MPIPSYKL_001547   ATAC      high       dex         2   20935.0 0.57
## 51 MPIPSYKL_001667   ATAC      high       veh         1   33561.5 0.38
## 52 MPIPSYKL_000637   ATAC       low       dex         2   84438.0 0.62
## 53 MPIPSYKL_001386   ATAC      high       dex         2   52700.0 0.59
## 54 MPIPSYKL_002468   ATAC      high       dex         2   92503.5 0.71
## 55 MPIPSYKL_002088   ATAC       low       dex         2  178600.5 0.63
## 56 MPIPSYKL_003013   ATAC      high       veh         1  101447.5 0.35
## 57 MPIPSYKL_000972   ATAC       low       dex         2  112234.5 0.43
## 58 MPIPSYKL_001048   ATAC      high       veh         1   95489.5 0.27
## 59 MPIPSYKL_001389   ATAC       low       veh         1   50559.5 0.44
## 60 MPIPSYKL_003785   ATAC      high       dex         2  378592.5 0.43
## 61 MPIPSYKL_003155   ATAC       low       veh         1   41054.5 0.36
## 62 MPIPSYKL_001886   ATAC       low       veh         1   98282.5 0.37
## 63 MPIPSYKL_003787   ATAC      high       veh         1   27634.0 0.45
## 64 MPIPSYKL_004246   ATAC      high       veh         1   49954.0 0.54
## 65 MPIPSYKL_002852   ATAC       low       veh         1   32191.5 0.47
## 66 MPIPSYKL_002613   ATAC      high       veh         1  199912.5 0.31
## 67 MPIPSYKL_001389   ATAC       low       dex         2  157832.0 0.74
## 68 MPIPSYKL_003155   ATAC       low       dex         2   77820.0 0.67
## 69 MPIPSYKL_000708   ATAC       low       dex         2   96744.0 0.57
## 70 MPIPSYKL_003911   ATAC       low       veh         1   18954.5 0.55
## 71 MPIPSYKL_001886   ATAC       low       dex         2  208394.5 0.48
## 72 MPIPSYKL_003013   ATAC      high       dex         2   62343.5 0.62
## 73 MPIPSYKL_002604   ATAC       low       veh         1   25425.5 0.47
## 74 MPIPSYKL_002533   ATAC      high       veh         1   41036.0 0.30
## 75 MPIPSYKL_001547   ATAC      high       veh         1   27024.5 0.43
## 76 MPIPSYKL_000988   ATAC      high       dex         2  200150.0 0.52
## 77 MPIPSYKL_004100   ATAC       low       veh         1   66633.5 0.39
## 78 MPIPSYKL_003472   ATAC      high       dex         2   37347.5 0.45
## 79 MPIPSYKL_001386   ATAC      high       veh         1   35902.5 0.42
## 80 MPIPSYKL_003911   ATAC       low       dex         2   30382.5 0.57
## 81 MPIPSYKL_003162   ATAC       low       veh         1   36747.0 0.39
## 82 MPIPSYKL_001701   ATAC      high       dex         2   95701.5 0.43
## 83 MPIPSYKL_002383   ATAC       low       dex         2   67016.0 0.46
## 84 MPIPSYKL_002658   ATAC       low       veh         1   21971.5 0.48
## 85 MPIPSYKL_001766   ATAC       low       dex         2  343078.0 0.33
## 86 MPIPSYKL_002852   ATAC       low       dex         2  576255.5 0.64
## 87 MPIPSYKL_001263   ATAC      high       dex         2  112898.5 0.69
## 88 MPIPSYKL_004198   ATAC       low       veh         1   19719.5 0.53
## 89 MPIPSYKL_003353   ATAC       low       dex         2  485290.5 0.52
## 90 MPIPSYKL_001667   ATAC      high       dex         2  158705.5 0.47
## 91 MPIPSYKL_002319   ATAC       low       dex         2   56561.0 0.58
## 92 MPIPSYKL_003785   ATAC      high       veh         1  217473.5 0.29
## 93 MPIPSYKL_001048   ATAC      high       dex         2  101095.5 0.60
## 94 MPIPSYKL_002095   ATAC       low       dex         2  809162.0 0.36
## 95 MPIPSYKL_002088   ATAC       low       veh         1  106472.0 0.33
##
## Design: [~Condition + Treatment] | 2 Contrasts:
##      Factor Group Samples Group2 Samples2
```

```
## 1 Condition   low      47   high       48
## 2 Treatment   dex      47    veh       48
```

8. Differential analysis :

8.1. Normalization

A windowing approach that identifies deferentially enriched regions (from csaw package)

```
# dba.contrast.norm <- dba.normalize(dba.contrast, normalize = DBA_NORM_LIB, library=DBA_LIBSIZE_PEAKRE
dba.contrast.norm <- dba.normalize(dba.contrast, normalize = DBA_NORM_LIB, background = T, method = DBA
dba.dif.anal.norm <- dba.analyze(dba.contrast.norm, method = DBA_ALL_METHODS)
```

```
dba.dif.anal.norm
```

```
## 95 Samples, 4781 sites in matrix (4784 total):
##                 ID Factor Condition Treatment Replicate     Reads FRiP
## 1  MPIPSYKL_000637   ATAC       low       veh         1   48646.5 0.48
## 2  MPIPSYKL_002418   ATAC       low       veh         1   39876.0 0.41
## 3  MPIPSYKL_003787   ATAC      high       dex         2  122369.5 0.60
## 4  MPIPSYKL_001701   ATAC      high       veh         1  252860.5 0.28
## 5  MPIPSYKL_003231   ATAC      high       veh         1   32347.0 0.38
## 6  MPIPSYKL_004171   ATAC       low       dex         2  173266.5 0.61
## 7  MPIPSYKL_002604   ATAC       low       dex         2   73592.0 0.63
## 8  MPIPSYKL_004054   ATAC       low       dex         2  133546.5 0.65
## 9  MPIPSYKL_001263   ATAC      high       veh         1   25906.5 0.46
## 10 MPIPSYKL_001786   ATAC      high       veh         1   38769.0 0.35
## 11 MPIPSYKL_001946   ATAC      high       veh         1   44678.0 0.41
## 12 MPIPSYKL_001113   ATAC      high       dex         2   26133.0 0.45
## 13 MPIPSYKL_000972   ATAC       low       veh         1   44139.0 0.42
## 14 MPIPSYKL_004100   ATAC       low       dex         2 1093528.0 0.38
## 15 MPIPSYKL_003331   ATAC       low       veh         1  136884.0 0.33
## 16 MPIPSYKL_003520   ATAC      high       veh         1   34792.5 0.37
## 17 MPIPSYKL_000988   ATAC      high       veh         1   97502.5 0.34
## 18 MPIPSYKL_004171   ATAC       low       veh         1   59254.5 0.34
## 19 MPIPSYKL_003331   ATAC       low       dex         2   80213.0 0.70
## 20 MPIPSYKL_001766   ATAC       low       veh         1   29695.5 0.35
## 21 MPIPSYKL_003520   ATAC      high       dex         2  137986.5 0.72
## 22 MPIPSYKL_004215   ATAC      high       dex         2  120145.0 0.63
## 23 MPIPSYKL_001946   ATAC      high       dex         2 1128909.5 0.51
## 24 MPIPSYKL_003353   ATAC       low       veh         1  136983.5 0.34
## 25 MPIPSYKL_002319   ATAC       low       veh         1   50702.5 0.50
## 26 MPIPSYKL_001109   ATAC      high       veh         1   89583.0 0.32
## 27 MPIPSYKL_004251   ATAC      high       veh         1   35730.5 0.42
## 28 MPIPSYKL_000708   ATAC       low       veh         1   33399.5 0.39
## 29 MPIPSYKL_004251   ATAC      high       dex         2   44901.0 0.41
## 30 MPIPSYKL_002802   ATAC       low       dex         2   79331.0 0.60
## 31 MPIPSYKL_002095   ATAC       low       veh         1   32884.0 0.44
## 32 MPIPSYKL_003162   ATAC       low       dex         2   41535.5 0.51
## 33 MPIPSYKL_002468   ATAC      high       veh         1   38420.0 0.42
## 34 MPIPSYKL_002658   ATAC       low       dex         2  315901.5 0.67
## 35 MPIPSYKL_003472   ATAC      high       veh         1   26032.0 0.47
## 36 MPIPSYKL_004054   ATAC       low       veh         1  174999.5 0.32
```

```
## 37 MPIPSYKL_002613   ATAC      high       dex         2   80250.5 0.49
## 38 MPIPSYKL_003231   ATAC      high       dex         2   88498.0 0.65
## 39 MPIPSYKL_002533   ATAC      high       dex         2  113871.0 0.45
## 40 MPIPSYKL_002802   ATAC       low       veh         1   52387.5 0.35
## 41 MPIPSYKL_003934   ATAC      high       veh         1   32213.5 0.39
## 42 MPIPSYKL_002418   ATAC       low       dex         2   56650.5 0.53
## 43 MPIPSYKL_001109   ATAC      high       dex         2   45694.0 0.56
## 44 MPIPSYKL_001786   ATAC      high       dex         2   65412.0 0.55
## 45 MPIPSYKL_003934   ATAC      high       dex         2  357234.0 0.60
## 46 MPIPSYKL_002383   ATAC       low       veh         1   38184.5 0.34
## 47 MPIPSYKL_004215   ATAC      high       veh         1   31424.0 0.48
## 48 MPIPSYKL_004246   ATAC      high       dex         2   86453.5 0.62
## 49 MPIPSYKL_001113   ATAC      high       veh         1   36781.5 0.35
## 50 MPIPSYKL_001547   ATAC      high       dex         2   20935.0 0.57
## 51 MPIPSYKL_001667   ATAC      high       veh         1   33561.5 0.38
## 52 MPIPSYKL_000637   ATAC       low       dex         2   84438.0 0.62
## 53 MPIPSYKL_001386   ATAC      high       dex         2   52700.0 0.59
## 54 MPIPSYKL_002468   ATAC      high       dex         2   92503.5 0.71
## 55 MPIPSYKL_002088   ATAC       low       dex         2  178600.5 0.63
## 56 MPIPSYKL_003013   ATAC      high       veh         1  101447.5 0.35
## 57 MPIPSYKL_000972   ATAC       low       dex         2  112234.5 0.43
## 58 MPIPSYKL_001048   ATAC      high       veh         1   95489.5 0.27
## 59 MPIPSYKL_001389   ATAC       low       veh         1   50559.5 0.44
## 60 MPIPSYKL_003785   ATAC      high       dex         2  378592.5 0.43
## 61 MPIPSYKL_003155   ATAC       low       veh         1   41054.5 0.36
## 62 MPIPSYKL_001886   ATAC       low       veh         1   98282.5 0.37
## 63 MPIPSYKL_003787   ATAC      high       veh         1   27634.0 0.45
## 64 MPIPSYKL_004246   ATAC      high       veh         1   49954.0 0.53
## 65 MPIPSYKL_002852   ATAC       low       veh         1   32191.5 0.47
## 66 MPIPSYKL_002613   ATAC      high       veh         1  199912.5 0.31
## 67 MPIPSYKL_001389   ATAC       low       dex         2  157832.0 0.74
## 68 MPIPSYKL_003155   ATAC       low       dex         2   77820.0 0.67
## 69 MPIPSYKL_000708   ATAC       low       dex         2   96744.0 0.57
## 70 MPIPSYKL_003911   ATAC       low       veh         1   18954.5 0.55
## 71 MPIPSYKL_001886   ATAC       low       dex         2  208394.5 0.48
## 72 MPIPSYKL_003013   ATAC      high       dex         2   62343.5 0.62
## 73 MPIPSYKL_002604   ATAC       low       veh         1   25425.5 0.47
## 74 MPIPSYKL_002533   ATAC      high       veh         1   41036.0 0.30
## 75 MPIPSYKL_001547   ATAC      high       veh         1   27024.5 0.43
## 76 MPIPSYKL_000988   ATAC      high       dex         2  200150.0 0.52
## 77 MPIPSYKL_004100   ATAC       low       veh         1   66633.5 0.39
## 78 MPIPSYKL_003472   ATAC      high       dex         2   37347.5 0.45
## 79 MPIPSYKL_001386   ATAC      high       veh         1   35902.5 0.42
## 80 MPIPSYKL_003911   ATAC       low       dex         2   30382.5 0.57
## 81 MPIPSYKL_003162   ATAC       low       veh         1   36747.0 0.39
## 82 MPIPSYKL_001701   ATAC      high       dex         2   95701.5 0.43
## 83 MPIPSYKL_002383   ATAC       low       dex         2   67016.0 0.46
## 84 MPIPSYKL_002658   ATAC       low       veh         1   21971.5 0.48
## 85 MPIPSYKL_001766   ATAC       low       dex         2  343078.0 0.33
## 86 MPIPSYKL_002852   ATAC       low       dex         2  576255.5 0.64
## 87 MPIPSYKL_001263   ATAC      high       dex         2  112898.5 0.69
## 88 MPIPSYKL_004198   ATAC       low       veh         1   19719.5 0.53
## 89 MPIPSYKL_003353   ATAC       low       dex         2  485290.5 0.52
## 90 MPIPSYKL_001667   ATAC      high       dex         2  158705.5 0.47
```

```
## 91 MPIPSYKL_002319   ATAC       low       dex         2   56561.0 0.58
## 92 MPIPSYKL_003785   ATAC      high       veh         1  217473.5 0.29
## 93 MPIPSYKL_001048   ATAC      high       dex         2  101095.5 0.60
## 94 MPIPSYKL_002095   ATAC       low       dex         2  809162.0 0.36
## 95 MPIPSYKL_002088   ATAC       low       veh         1  106472.0 0.33
##
## Design: [~Condition + Treatment] | 2 Contrasts:
##      Factor Group Samples Group2 Samples2 DB.edgeR DB.DESeq2
## 1 Condition   low      47   high       48        1         0
## 2 Treatment   dex      47    veh       48     3285      1809
```

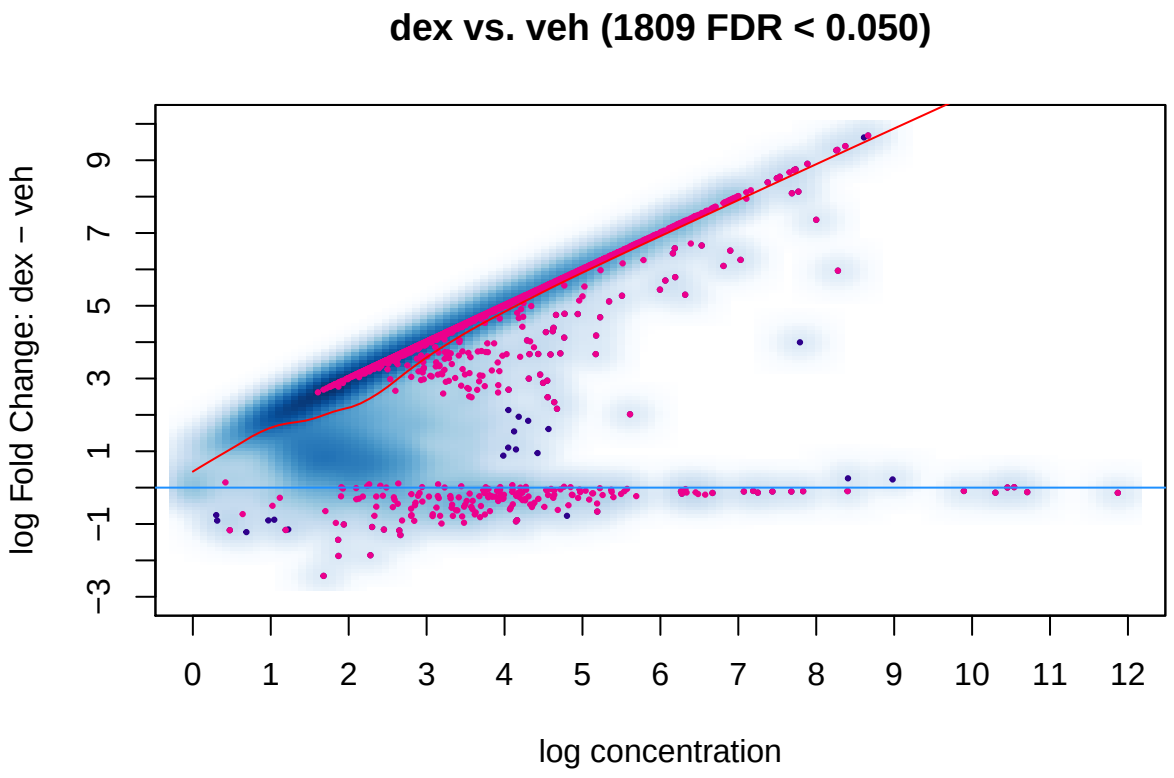
Result :

FDR = 5% :

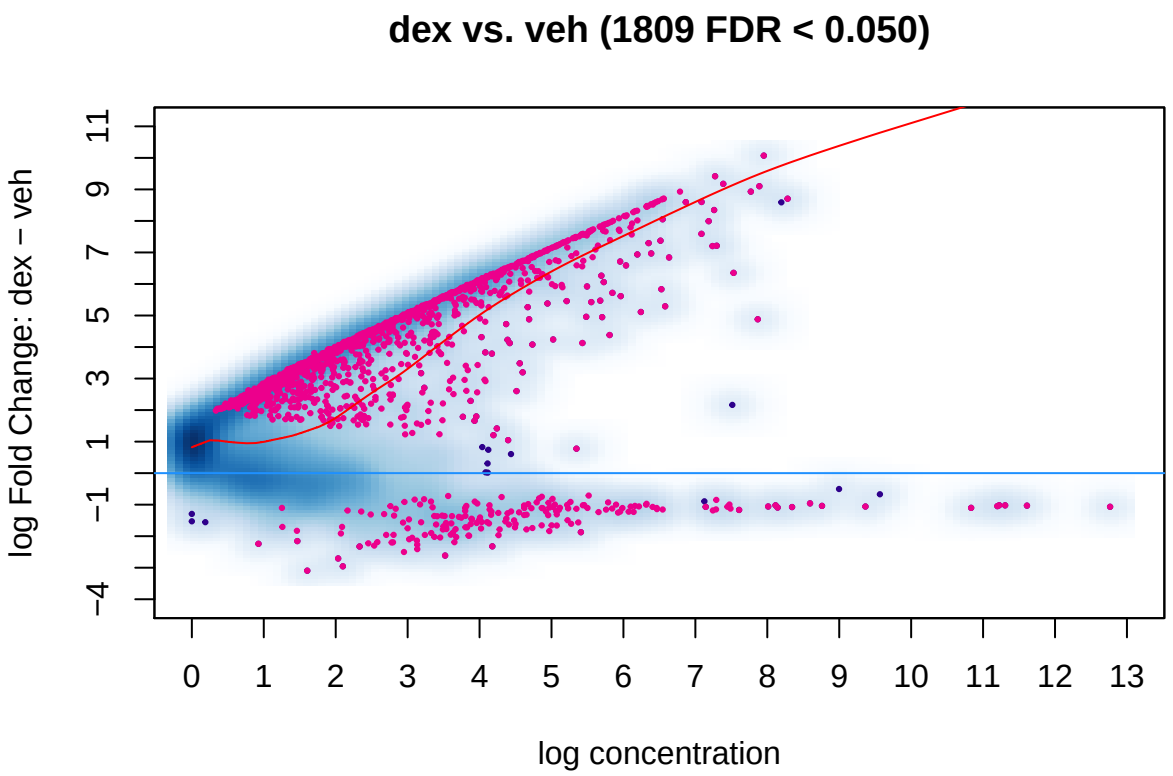
DeSeq : 1809 of the 4781 are identified as being significantly differentially bound

EdgeR : 3285 of the 4781

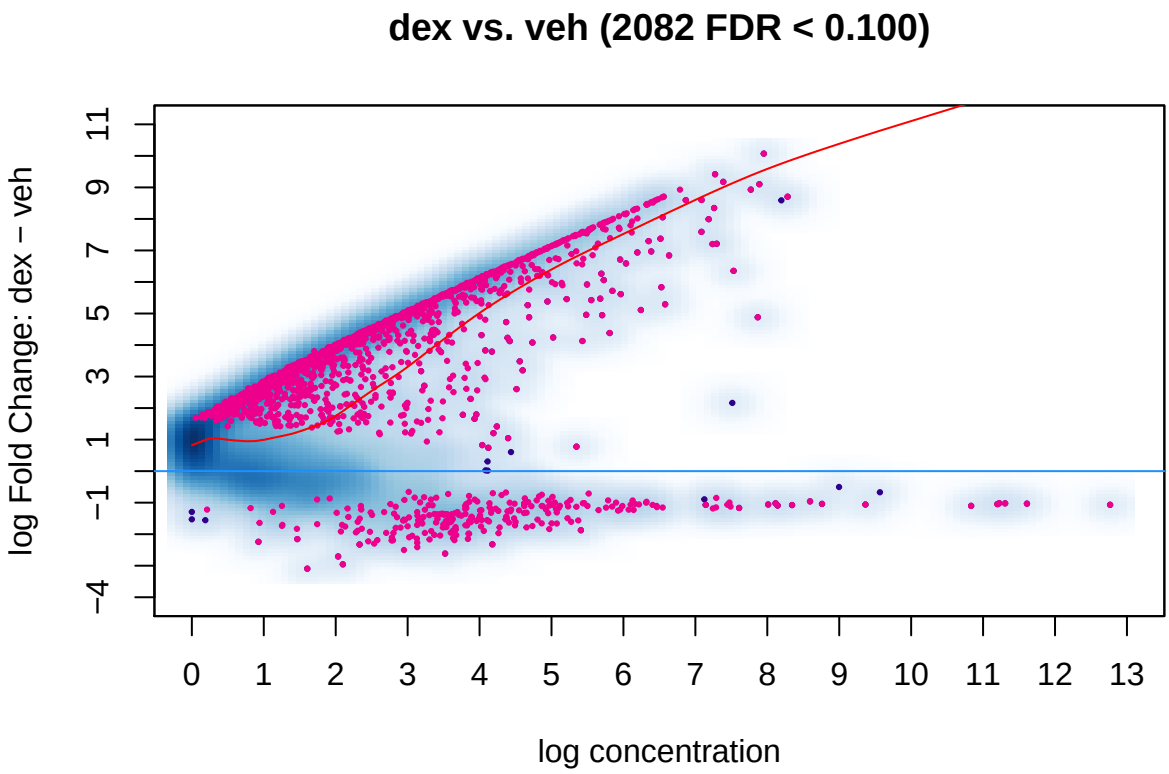
```
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2, bNormalized=F)
```




```
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2)
```




```
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2, th = 0.1)
```



8.2. In-built normalization

```
dba.dif.anal <- dba.analyze(dba.contrast, method = DBA_ALL_METHODS)
```

```
## Applying Blacklist/Greylists...

## Genome detected: Hsapiens.NCBI.GRCh38

## Applying blacklist...

## Removed: 3 of 4784 intervals.

## No control reads specified, unable to generate greylist.

## Removed 3 (of 4784) consensus peaks.

## Normalize edgeR with defaults...

## Normalize DESeq2 with defaults...

## Analyzing...
```

```
dba.dif.anal
```

```
## 95 Samples, 4781 sites in matrix (4784 total):
##                 ID Factor Condition Treatment Replicate     Reads FRiP
## 1  MPIPSYKL_000637   ATAC       low       veh         1   48646.5 0.48
## 2  MPIPSYKL_002418   ATAC       low       veh         1   39876.0 0.41
## 3  MPIPSYKL_003787   ATAC      high       dex         2  122369.5 0.60
## 4  MPIPSYKL_001701   ATAC      high       veh         1  252860.5 0.28
## 5  MPIPSYKL_003231   ATAC      high       veh         1   32347.0 0.38
## 6  MPIPSYKL_004171   ATAC       low       dex         2  173266.5 0.61
## 7  MPIPSYKL_002604   ATAC       low       dex         2   73592.0 0.63
## 8  MPIPSYKL_004054   ATAC       low       dex         2  133546.5 0.65
## 9  MPIPSYKL_001263   ATAC      high       veh         1   25906.5 0.46
## 10 MPIPSYKL_001786   ATAC      high       veh         1   38769.0 0.35
## 11 MPIPSYKL_001946   ATAC      high       veh         1   44678.0 0.41
## 12 MPIPSYKL_001113   ATAC      high       dex         2   26133.0 0.45
## 13 MPIPSYKL_000972   ATAC       low       veh         1   44139.0 0.42
## 14 MPIPSYKL_004100   ATAC       low       dex         2 1093528.0 0.38
## 15 MPIPSYKL_003331   ATAC       low       veh         1  136884.0 0.33
## 16 MPIPSYKL_003520   ATAC      high       veh         1   34792.5 0.37
## 17 MPIPSYKL_000988   ATAC      high       veh         1   97502.5 0.34
## 18 MPIPSYKL_004171   ATAC       low       veh         1   59254.5 0.34
## 19 MPIPSYKL_003331   ATAC       low       dex         2   80213.0 0.70
## 20 MPIPSYKL_001766   ATAC       low       veh         1   29695.5 0.35
## 21 MPIPSYKL_003520   ATAC      high       dex         2  137986.5 0.72
## 22 MPIPSYKL_004215   ATAC      high       dex         2  120145.0 0.63
## 23 MPIPSYKL_001946   ATAC      high       dex         2 1128909.5 0.51
## 24 MPIPSYKL_003353   ATAC       low       veh         1  136983.5 0.34
## 25 MPIPSYKL_002319   ATAC       low       veh         1   50702.5 0.50
## 26 MPIPSYKL_001109   ATAC      high       veh         1   89583.0 0.32
## 27 MPIPSYKL_004251   ATAC      high       veh         1   35730.5 0.42
## 28 MPIPSYKL_000708   ATAC       low       veh         1   33399.5 0.39
## 29 MPIPSYKL_004251   ATAC      high       dex         2   44901.0 0.41
## 30 MPIPSYKL_002802   ATAC       low       dex         2   79331.0 0.60
## 31 MPIPSYKL_002095   ATAC       low       veh         1   32884.0 0.44
## 32 MPIPSYKL_003162   ATAC       low       dex         2   41535.5 0.51
## 33 MPIPSYKL_002468   ATAC      high       veh         1   38420.0 0.42
## 34 MPIPSYKL_002658   ATAC       low       dex         2  315901.5 0.67
## 35 MPIPSYKL_003472   ATAC      high       veh         1   26032.0 0.47
## 36 MPIPSYKL_004054   ATAC       low       veh         1  174999.5 0.32
## 37 MPIPSYKL_002613   ATAC      high       dex         2   80250.5 0.49
## 38 MPIPSYKL_003231   ATAC      high       dex         2   88498.0 0.65
## 39 MPIPSYKL_002533   ATAC      high       dex         2  113871.0 0.45
## 40 MPIPSYKL_002802   ATAC       low       veh         1   52387.5 0.35
## 41 MPIPSYKL_003934   ATAC      high       veh         1   32213.5 0.39
## 42 MPIPSYKL_002418   ATAC       low       dex         2   56650.5 0.53
## 43 MPIPSYKL_001109   ATAC      high       dex         2   45694.0 0.56
## 44 MPIPSYKL_001786   ATAC      high       dex         2   65412.0 0.55
## 45 MPIPSYKL_003934   ATAC      high       dex         2  357234.0 0.60
## 46 MPIPSYKL_002383   ATAC       low       veh         1   38184.5 0.34
## 47 MPIPSYKL_004215   ATAC      high       veh         1   31424.0 0.48
## 48 MPIPSYKL_004246   ATAC      high       dex         2   86453.5 0.62
## 49 MPIPSYKL_001113   ATAC      high       veh         1   36781.5 0.35
## 50 MPIPSYKL_001547   ATAC      high       dex         2   20935.0 0.57
```

```
## 51 MPIPSYKL_001667   ATAC      high       veh         1   33561.5 0.38
## 52 MPIPSYKL_000637   ATAC       low       dex         2   84438.0 0.62
## 53 MPIPSYKL_001386   ATAC      high       dex         2   52700.0 0.59
## 54 MPIPSYKL_002468   ATAC      high       dex         2   92503.5 0.71
## 55 MPIPSYKL_002088   ATAC       low       dex         2  178600.5 0.63
## 56 MPIPSYKL_003013   ATAC      high       veh         1  101447.5 0.35
## 57 MPIPSYKL_000972   ATAC       low       dex         2  112234.5 0.43
## 58 MPIPSYKL_001048   ATAC      high       veh         1   95489.5 0.27
## 59 MPIPSYKL_001389   ATAC       low       veh         1   50559.5 0.44
## 60 MPIPSYKL_003785   ATAC      high       dex         2  378592.5 0.43
## 61 MPIPSYKL_003155   ATAC       low       veh         1   41054.5 0.36
## 62 MPIPSYKL_001886   ATAC       low       veh         1   98282.5 0.37
## 63 MPIPSYKL_003787   ATAC      high       veh         1   27634.0 0.45
## 64 MPIPSYKL_004246   ATAC      high       veh         1   49954.0 0.53
## 65 MPIPSYKL_002852   ATAC       low       veh         1   32191.5 0.47
## 66 MPIPSYKL_002613   ATAC      high       veh         1  199912.5 0.31
## 67 MPIPSYKL_001389   ATAC       low       dex         2  157832.0 0.74
## 68 MPIPSYKL_003155   ATAC       low       dex         2   77820.0 0.67
## 69 MPIPSYKL_000708   ATAC       low       dex         2   96744.0 0.57
## 70 MPIPSYKL_003911   ATAC       low       veh         1   18954.5 0.55
## 71 MPIPSYKL_001886   ATAC       low       dex         2  208394.5 0.48
## 72 MPIPSYKL_003013   ATAC      high       dex         2   62343.5 0.62
## 73 MPIPSYKL_002604   ATAC       low       veh         1   25425.5 0.47
## 74 MPIPSYKL_002533   ATAC      high       veh         1   41036.0 0.30
## 75 MPIPSYKL_001547   ATAC      high       veh         1   27024.5 0.43
## 76 MPIPSYKL_000988   ATAC      high       dex         2  200150.0 0.52
## 77 MPIPSYKL_004100   ATAC       low       veh         1   66633.5 0.39
## 78 MPIPSYKL_003472   ATAC      high       dex         2   37347.5 0.45
## 79 MPIPSYKL_001386   ATAC      high       veh         1   35902.5 0.42
## 80 MPIPSYKL_003911   ATAC       low       dex         2   30382.5 0.57
## 81 MPIPSYKL_003162   ATAC       low       veh         1   36747.0 0.39
## 82 MPIPSYKL_001701   ATAC      high       dex         2   95701.5 0.43
## 83 MPIPSYKL_002383   ATAC       low       dex         2   67016.0 0.46
## 84 MPIPSYKL_002658   ATAC       low       veh         1   21971.5 0.48
## 85 MPIPSYKL_001766   ATAC       low       dex         2  343078.0 0.33
## 86 MPIPSYKL_002852   ATAC       low       dex         2  576255.5 0.64
## 87 MPIPSYKL_001263   ATAC      high       dex         2  112898.5 0.69
## 88 MPIPSYKL_004198   ATAC       low       veh         1   19719.5 0.53
## 89 MPIPSYKL_003353   ATAC       low       dex         2  485290.5 0.52
## 90 MPIPSYKL_001667   ATAC      high       dex         2  158705.5 0.47
## 91 MPIPSYKL_002319   ATAC       low       dex         2   56561.0 0.58
## 92 MPIPSYKL_003785   ATAC      high       veh         1  217473.5 0.29
## 93 MPIPSYKL_001048   ATAC      high       dex         2  101095.5 0.60
## 94 MPIPSYKL_002095   ATAC       low       dex         2  809162.0 0.36
## 95 MPIPSYKL_002088   ATAC       low       veh         1  106472.0 0.33
##
## Design: [~Condition + Treatment] | 2 Contrasts:
##      Factor Group Samples Group2 Samples2 DB.edgeR DB.DESeq2
## 1 Condition   low      47   high       48        4         0
## 2 Treatment   dex      47    veh       48     3270      1766
```

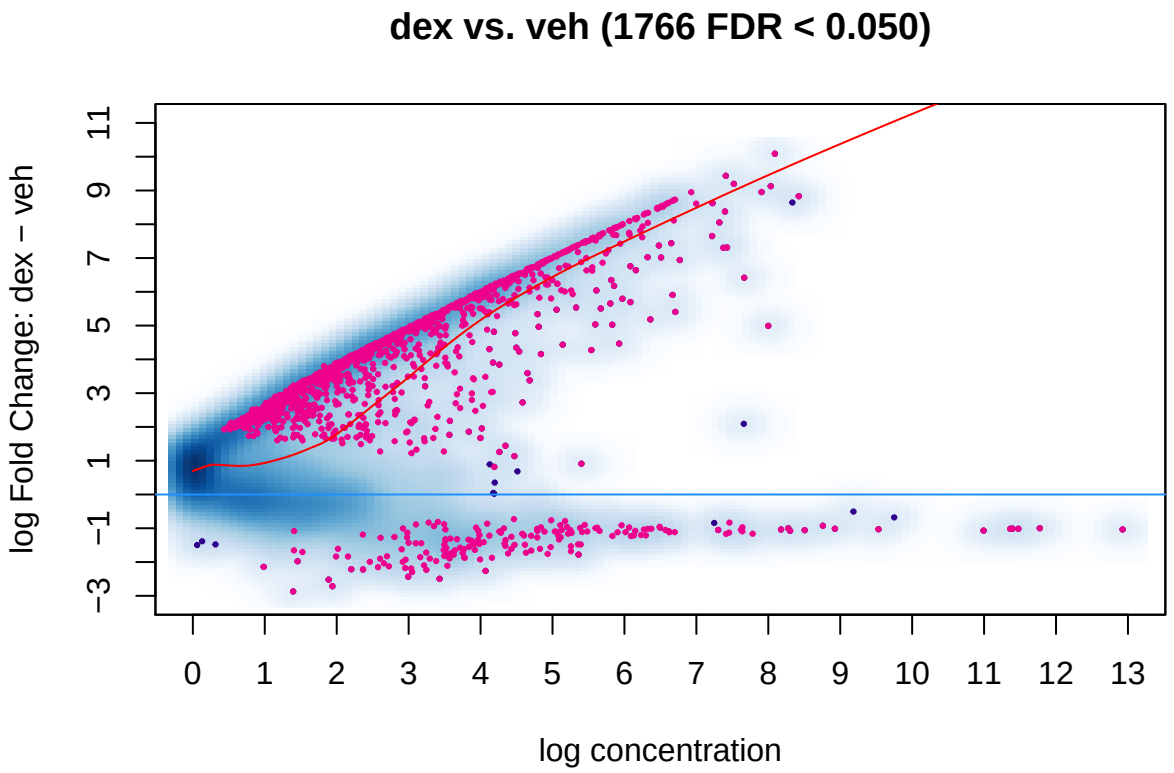
Result :

FDR = 5% :

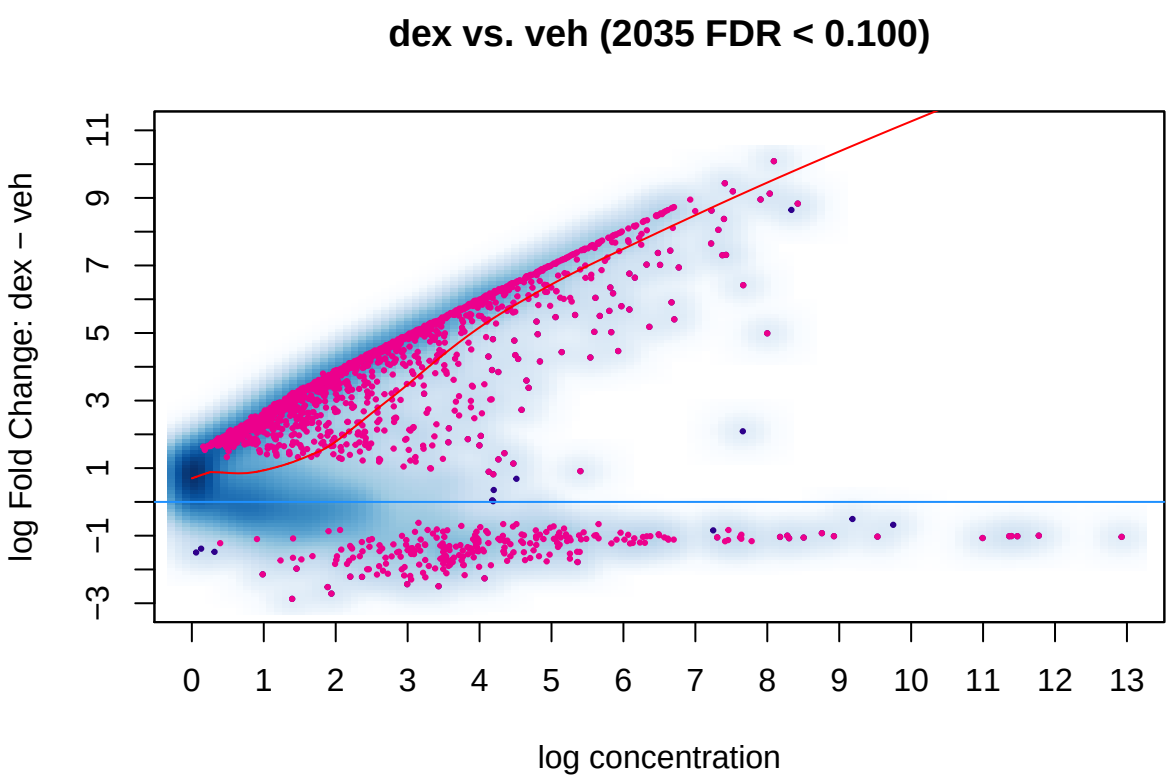
DeSeq : 1766 of the 4781 are identified as being significantly differentially bound

EdgeR : 3270 of the 4781

```
dba.plotMA(dba.dif.anal, method = DBA_DESEQ2, contrast = 2, bNormalized=T)
```

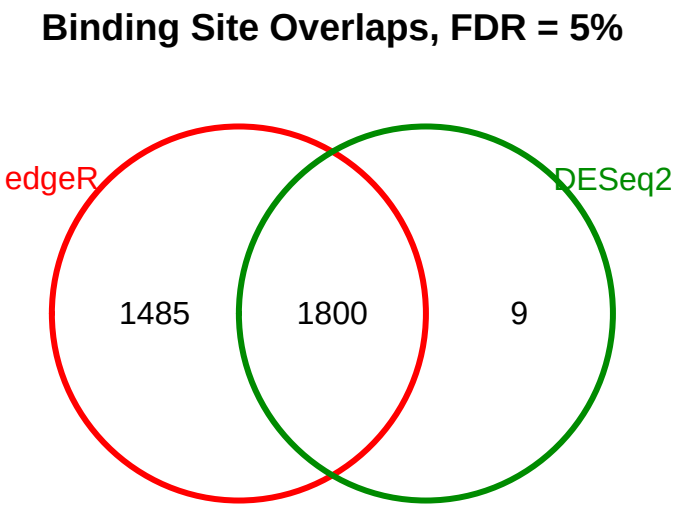



```
dba.plotMA(dba.dif.anal, method = DBA_DESEQ2, contrast = 2, bNormalized = T, th = 0.1)
```



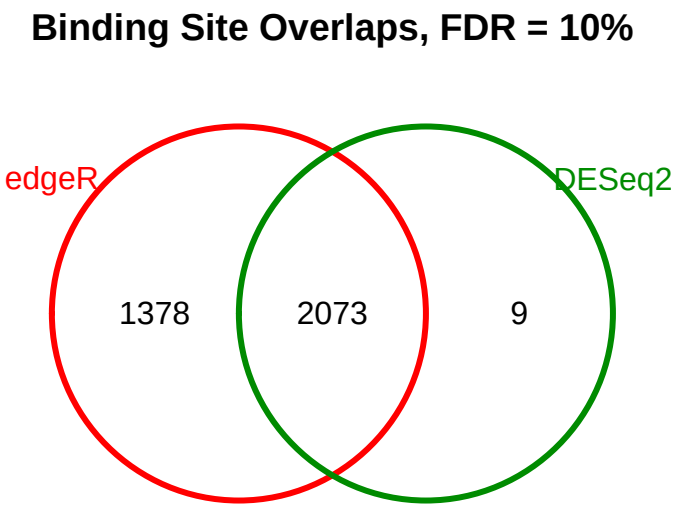

```
dba.dif.anal <- dba.dif.anal.norm
```

```
dba.plotVenn(dba.dif.anal, contrast = 2, method = DBA_ALL_METHODS, main = "Binding Site Overlaps, FDR =
```



dex vs. veh:DB:All

```
# dba.dif.anal.th.01 <- dba.dif.anal
dba.dif.anal$config$th <- 0.10
dba.plotVenn(dba.dif.anal, contrast = 2, method = DBA_ALL_METHODS, main = "Binding Site Overlaps, FDR =
```



dex vs. veh:DB:All

```
# dba.dif.anal.overlap <- dba.overlap(dba.dif.anal, mode = DBA_OLAP_PEAKS,
#                             contrast = 2, method=dba.dif.anal$config$AnalysisMethod, th = dba.dif.anal$
#                             bUsePval = dba.dif.anal$config$bUsePval,
#                             bCorOnly=TRUE, CorMethod="pearson",
#                             DataType = dba.dif.anal$config$DataType)

dba.dif.anal.deseq <- dba.dif.anal$DESeq2
# dba.dif.anal.deseq@assays@data@listData$counts

dba.dif.anal.deseq <- dba.analyze(dba.contrast, method = DBA_DESEQ2)
dba.dif.anal.deseq$config$th <- 0.10
```

9. Retrieve the differentially bound sites

```
dex.report <- dba.report(dba.dif.anal.deseq, contrast = 2, method = DBA_DESEQ2, th = dba.dif.anal.deseq$
                                                bCounts = T, bCalled = T) # file = paste0(result.dir.fn
write.csv2(dex.report, file = paste0(result.dir.fn, "dex_deseq_report.csv"), row.names = F)

# dex.report <- read.csv2(paste0(result.dir.fn, "dex_deseq_report.csv"))

head(dex.report)
```

```
## GRanges object with 6 ranges and 101 metadata columns:
##        seqnames              ranges strand |      Conc  Conc_dex  Conc_veh
```

```
##          <Rle>          <IRanges>  <Rle> | <numeric> <numeric> <numeric>
##   4102       8   11545707-11546107      * |      7.41      8.43         0
##   1431      15   31365735-31366135      * |      8.09      9.11         0
##   2274       2   43131375-43131775      * |      7.52      8.54         0
##   3688       6   35727909-35728309      * |      8.42      9.44         0
##   3577       6   12369970-12370370      * |      6.93      7.94         0
##   2500       2 201126779-201127179      * |      7.22      8.24         0
##           Fold   p-value       FDR MPIPSYKL_003787 MPIPSYKL_004171
##      <numeric> <numeric> <numeric>       <numeric>       <numeric>
##   4102      9.43 5.67e-194 2.16e-190          360.28          397.85
##   1431     10.09 4.17e-186 6.92e-183          688.27          589.05
##   2274      9.19 5.44e-186 6.92e-183          346.74          352.25
##   3688      8.83 7.96e-179 7.59e-176          740.33          822.90
##   3577      8.95 1.69e-173 1.29e-170          306.13          194.14
##   2500      8.63 3.64e-165 2.31e-162          352.99          330.19
##        MPIPSYKL_002604 MPIPSYKL_004054 MPIPSYKL_001113 MPIPSYKL_004100
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          351.48          408.36          180.40          126.77
##   1431          843.20          729.89          468.07          245.51
##   2274          592.14          462.74          307.17          204.73
##   3688          818.96          747.07          336.43          197.27
##   3577          244.13          282.42          151.15           78.53
##   2500          335.89          365.42          204.78          119.43
##        MPIPSYKL_003331 MPIPSYKL_003520 MPIPSYKL_004215 MPIPSYKL_001946
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          548.03          447.85          403.00          284.77
##   1431          899.09          649.16          605.57          419.98
##   2274          624.28          409.07          395.58          311.07
##   3688         1146.90          801.52          712.68          471.90
##   3577          409.83          363.82          338.31          177.99
##   2500          465.43          433.08          373.31          235.67
##        MPIPSYKL_004251 MPIPSYKL_002802 MPIPSYKL_003162 MPIPSYKL_002658
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          246.88          460.97          380.39          485.23
##   1431          340.53          634.43          613.54          732.48
##   2274          204.32          531.64          435.61          539.68
##   3688          476.74          812.72          776.13          812.75
##   3577          187.29          295.53          242.35          229.10
##   2500          190.13          300.35          303.70          342.85
##        MPIPSYKL_002613 MPIPSYKL_003231 MPIPSYKL_002533 MPIPSYKL_002418
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          266.74          388.74          261.84          290.15
##   1431          485.85          643.58          459.90          494.82
##   2274          349.31          368.59          320.03          296.89
##   3688          687.50          799.08          778.80          616.28
##   3577          242.93          407.46          189.11          157.44
##   2500          273.09          374.34          217.08          283.40
##        MPIPSYKL_001109 MPIPSYKL_001786 MPIPSYKL_003934 MPIPSYKL_004246
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          404.33          383.74          368.45          456.89
##   1431          680.40          656.45          521.47          627.85
##   2274          479.62          440.23          339.56          481.94
##   3688          839.34          695.41          694.81          854.82
##   3577          317.89          340.89          217.93          266.76
```

```
##   2500          342.99          350.63          303.18          330.14
##        MPIPSYKL_001547 MPIPSYKL_000637 MPIPSYKL_001386 MPIPSYKL_002468
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          383.44          452.70          427.95          608.83
##   1431          748.62          872.21          512.57          807.18
##   2274          450.39          520.61          343.33          508.28
##   3688          906.87          932.57          853.48         1141.90
##   3577          353.01          307.84          263.54          422.87
##   2500          365.18          362.16          435.20          487.61
##        MPIPSYKL_002088 MPIPSYKL_000972 MPIPSYKL_003785 MPIPSYKL_001389
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          341.02          133.96          150.44          502.95
##   1431          515.81          199.81          199.24          676.52
##   2274          266.82          136.23          137.99          423.83
##   3688          659.21          291.77          276.31          906.60
##   3577          222.59           88.55          118.13          309.20
##   2500          315.33          124.88          114.09          460.16
##        MPIPSYKL_003155 MPIPSYKL_000708 MPIPSYKL_001886 MPIPSYKL_003013
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          509.21          392.49          194.43          337.23
##   1431          800.66          728.34            0.00          676.50
##   2274          469.92          500.48          199.33          468.03
##   3688         1000.42          899.56          369.30          913.58
##   3577          350.39          345.07          132.68          296.35
##   2500          438.81          384.58          185.26          400.59
##        MPIPSYKL_000988 MPIPSYKL_003472 MPIPSYKL_003911 MPIPSYKL_001701
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          234.27          238.82          415.19          145.12
##   1431          442.45          426.46          658.43          179.74
##   2274          270.56          252.47          410.99          149.12
##   3688          588.23          583.40          855.54          210.36
##   3577          211.99          272.94          247.43          109.18
##   2500          255.28          262.70          301.95          121.16
##        MPIPSYKL_002383 MPIPSYKL_001766 MPIPSYKL_002852 MPIPSYKL_001263
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          214.85           98.42          352.90          633.15
##   1431          539.97          103.99          607.84          939.00
##   2274          382.16           77.25          552.79          692.97
##   3688          751.02          177.53          688.11         1049.61
##   3577          205.34           59.42          262.02          446.93
##   2500          256.68           73.54          335.43          462.73
##        MPIPSYKL_003353 MPIPSYKL_001667 MPIPSYKL_002319 MPIPSYKL_001048
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          259.67          191.88          457.31          420.97
##   1431          391.74          240.05          675.83          702.03
##   2274          254.42          160.57          410.00          442.39
##   3688          518.03          368.51          806.49          902.43
##   3577          159.11          147.73          283.85          234.43
##   2500          235.25          175.83          346.92          334.00
##        MPIPSYKL_002095 MPIPSYKL_000637 MPIPSYKL_002418 MPIPSYKL_001701
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102          154.16               0               0            0.00
##   1431          242.19               0               0            0.00
##   2274          171.48               0               0            0.00
```

```
##   3688          292.74               0               0            3.53
##   3577           85.51               0               0            0.00
##   2500          127.71               0               0            0.00
##        MPIPSYKL_003231 MPIPSYKL_001263 MPIPSYKL_001786 MPIPSYKL_001946
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0               0
##   1431               0               0               0               0
##   2274               0               0               0               0
##   3688               0               0               0               0
##   3577               0               0               0               0
##   2500               0               0               0               0
##        MPIPSYKL_000972 MPIPSYKL_003331 MPIPSYKL_003520 MPIPSYKL_000988
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0               0
##   1431               0               0               0               0
##   2274               0               0               0               0
##   3688               0               0               0               0
##   3577               0               0               0               0
##   2500               0               0               0               0
##        MPIPSYKL_004171 MPIPSYKL_001766 MPIPSYKL_003353 MPIPSYKL_002319
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0            0.00               0
##   1431               0               0            0.00               0
##   2274               0               0            0.00               0
##   3688               0               0           16.74               0
##   3577               0               0            0.00               0
##   2500               0               0            0.00               0
##        MPIPSYKL_001109 MPIPSYKL_004251 MPIPSYKL_000708 MPIPSYKL_002095
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0               0
##   1431               0               0               0               0
##   2274               0               0               0               0
##   3688               0               0               0               0
##   3577               0               0               0               0
##   2500               0               0               0               0
##        MPIPSYKL_002468 MPIPSYKL_003472 MPIPSYKL_004054 MPIPSYKL_002802
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0            0.00               0
##   1431               0               0            0.00               0
##   2274               0               0            0.00               0
##   3688               0               0            3.64               0
##   3577               0               0            0.00               0
##   2500               0               0            0.00               0
##        MPIPSYKL_003934 MPIPSYKL_002383 MPIPSYKL_004215 MPIPSYKL_001113
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0               0
##   1431               0               0               0               0
##   2274               0               0               0               0
##   3688               0               0               0               0
##   3577               0               0               0               0
##   2500               0               0               0               0
##        MPIPSYKL_001667 MPIPSYKL_003013 MPIPSYKL_001048 MPIPSYKL_001389
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0               0
```

```
##   1431              0              0              0              0
##   2274              0              0              0              0
##   3688              0              0              0              0
##   3577              0              0              0              0
##   2500              0              0              0              0
##        MPIPSYKL_003155 MPIPSYKL_001886 MPIPSYKL_003787 MPIPSYKL_004246
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0            0.00               0              0
##   1431               0            0.00               0              0
##   2274               0            7.78               0              0
##   3688               0           14.26               0              0
##   3577               0            0.00               0              0
##   2500               0           14.26               0              0
##        MPIPSYKL_002852 MPIPSYKL_002613 MPIPSYKL_003911 MPIPSYKL_002604
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0              0
##   1431               0               0               0              0
##   2274               0               0               0              0
##   3688               0               0               0              0
##   3577               0               0               0              0
##   2500               0               0               0              0
##        MPIPSYKL_002533 MPIPSYKL_001547 MPIPSYKL_004100 MPIPSYKL_001386
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0              0
##   1431               0               0               0              0
##   2274               0               0               0              0
##   3688               0               0               0              0
##   3577               0               0               0              0
##   2500               0               0               0              0
##        MPIPSYKL_003162 MPIPSYKL_002658 MPIPSYKL_004198 MPIPSYKL_003785
##              <numeric>       <numeric>       <numeric>       <numeric>
##   4102               0               0               0              0
##   1431               0               0               0              0
##   2274               0               0               0              0
##   3688               0               0               0              0
##   3577               0               0               0              0
##   2500               0               0               0              0
##        MPIPSYKL_002088
##              <numeric>
##   4102               0
##   1431               0
##   2274               0
##   3688               0
##   3577               0
##   2500               0
##   -------
##   seqinfo: 80 sequences from an unspecified genome; no seqlengths
```

- "Conc" shows the mean read concentration over all the samples (the default calculation uses log2 normalized read counts)
- "Conc_dex" - the mean concentration over the samples in dex group
- "Conc_veh" - the mean concentration over the samples in veh group.
- "Fold" - the log fold changes (LFCs) between the two groups. A positive value indicates increased binding affinity in the DEX group, and a negative value indicates increased binding affinity in the

VEH group.
- normalized count data for individual samples

The number of differentially bound sites that have enriched in DEX samples :

```
sum(dex.report$Fold > 0)
```

```
## [1] 1784
```

The number of differentially bound sites that have enriched in VEH samples :

```
sum(dex.report$Fold < 0)
```

```
## [1] 251
```

10. Quantification for edgeR

```
counts.edger <- dba.dif.anal$edgeR$DEdata$counts
```

11. Peaks annotation

```
#  This is the refeernce anno used: /ngs/references/human/GRCh38/Homo_sapiens.GRCh38.97.gtf
ref.annot.fn <- paste(wd, "Homo_sapiens.GRCh38.97.gtf", sep = "/")

txDb <- makeTxDbFromGFF(ref.annot.fn, format = "gtf",
                            organism = "Homo sapiens",
                            chrominfo = NULL,
                            dbxrefTag = "gene_id")
```

```
## Import genomic features from the file as a GRanges object ... OK
## Prepare the 'metadata' data frame ... OK
## Make the TxDb object ...
```

```
## Warning in .get_cds_IDX(mcols0$type, mcols0$phase): The "phase" metadata column contains non-NA valu
##   stop_codon. This information was ignored.
```

```
## OK
```

```
# annotate all peaks
dex.anno.report <- ChIPseeker::annotatePeak(dex.report, tssRegion = c(-1000, 1000), TxDb=txDb, verbose =
```

```
## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the oth
##   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI27
##   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI27
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).
```

```
## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI27
##   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI27
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI27
##   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI27
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI27
##   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI27
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI27
##   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI27
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).
```

```
dex.anno.report
```

```
## Annotated peaks generated by ChIPseeker
## 1961/2035  peaks were annotated
## Genomic Annotation Summary:
##              Feature  Frequency
## 9           Promoter 20.7037226
## 4             5' UTR  0.1019888
## 3             3' UTR  2.0397756
## 1           1st Exon  0.2039776
## 7         Other Exon  2.3457420
## 2         1st Intron 18.3069862
## 8       Other Intron 28.5058644
## 6 Downstream (<=300)  1.9887812
## 5  Distal Intergenic 25.8031617
```

12. Export data

12.1. Export annotated data

```
annotation.df   <- as.data.frame(dex.anno.report@anno)
write.csv2(annotation.df, file = paste0(result.dir.fn, "dex_deseq_report_annotated.csv"), row.names = F

anno.rasqual.df <- annotation.df[,c(113, 12:106)] # take only geneID and sampleIDs
write.csv2(anno.rasqual.df, file = paste0(result.dir.fn, "dex_deseq_anno_count_mtrx_for_rasqual.csv"), 
```

12.2 Export only dex and veh enriched results

Write files for each set of significant regions identified by DeSeq, separating them based on the gain or loss of enrichment.

DEX:

```
dex.enrich.df <- count.mtrx.df  %>% filter(FDR < 0.1 & Fold > 0)
write.csv2(dex.enrich.df, file = paste0(result.dir.fn, "dex_deseq_report_only_dex_echriched.csv"), row.

veh.enrich.df <- count.mtrx.df  %>% filter(FDR < 0.1 & Fold < 0)
write.csv2(veh.enrich.df, file = paste0(result.dir.fn, "dex_deseq_report_only_veh_echriched.csv"), row.
```

12.3. Export count matrices for RASQUAL

DEX:

```
count.mtrx.dex.rasqual <- read.csv2(paste0(result.dir.fn, "count_mtrx_dex_for_rasqual.csv"))
dex.samples <- c("PEAKSET_ID", c(read.table(paste0(result.dir.fn, "chipseq_dex_samples.txt")))[[1]])
count.mtrx.dex.rasqual.out <- count.mtrx.dex.rasqual[dex.samples]

write.table(count.mtrx.dex.rasqual[2:nrow(count.mtrx.dex.rasqual.out), ], paste0(result.dir.fn, "count_

write.table(count.mtrx.dex.rasqual[2:nrow(count.mtrx.dex.rasqual.out), ], paste0("/Users/anastasiia_hry
```

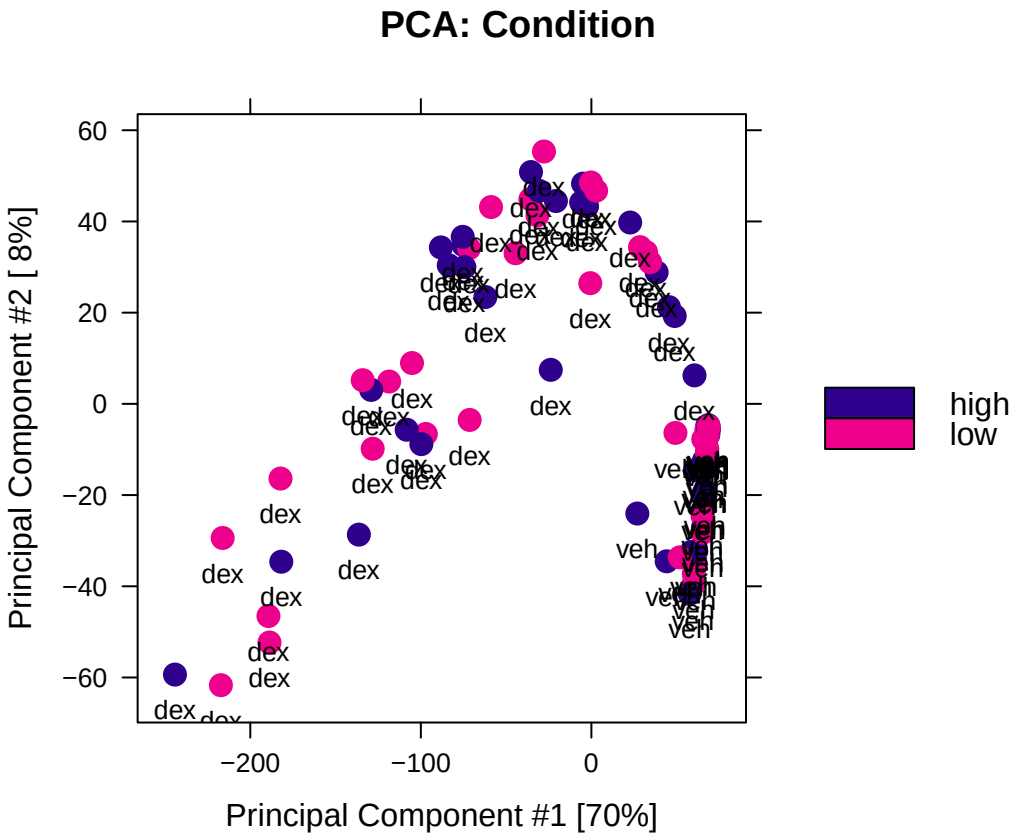
VEH:

```
# count.mtrx.veh.rasqual <- read.csv2(paste0(result.dir.fn, "count_mtrx_veh_for_rasqual_2.csv"))
# veh.samples <- c("PEAKSET_ID", c(read.table(paste0(result.dir.fn, "chipseq_veh_samples.txt")))[[1]])
# count.mtrx.veh.rasqual.out <- count.mtrx.dex.rasqual[veh.samples]
#
# write.table(count.mtrx.veh.rasqual[2:nrow(count.mtrx.veh.rasqual.out), ], paste0(result.dir.fn, "coun
#
# write.table(count.mtrx.veh.rasqual[2:nrow(count.mtrx.veh.rasqual.out), ], paste0("/Users/anastasiia_h
```

13. Plotting

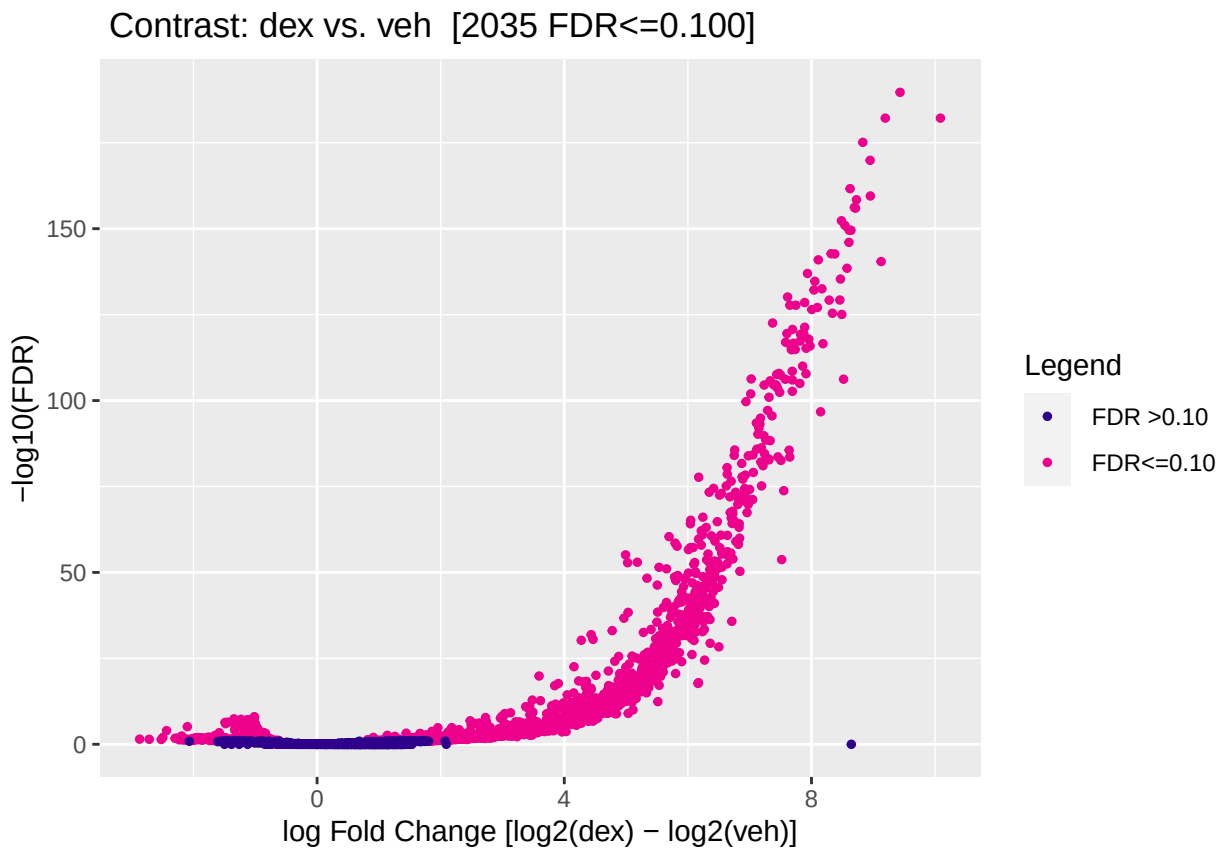
13.1 PCA

```
dba.plotPCA(dba.dif.anal.deseq, method = DBA_DESEQ2, attributes = DBA_CONDITION, label = DBA_TREATMENT,
```



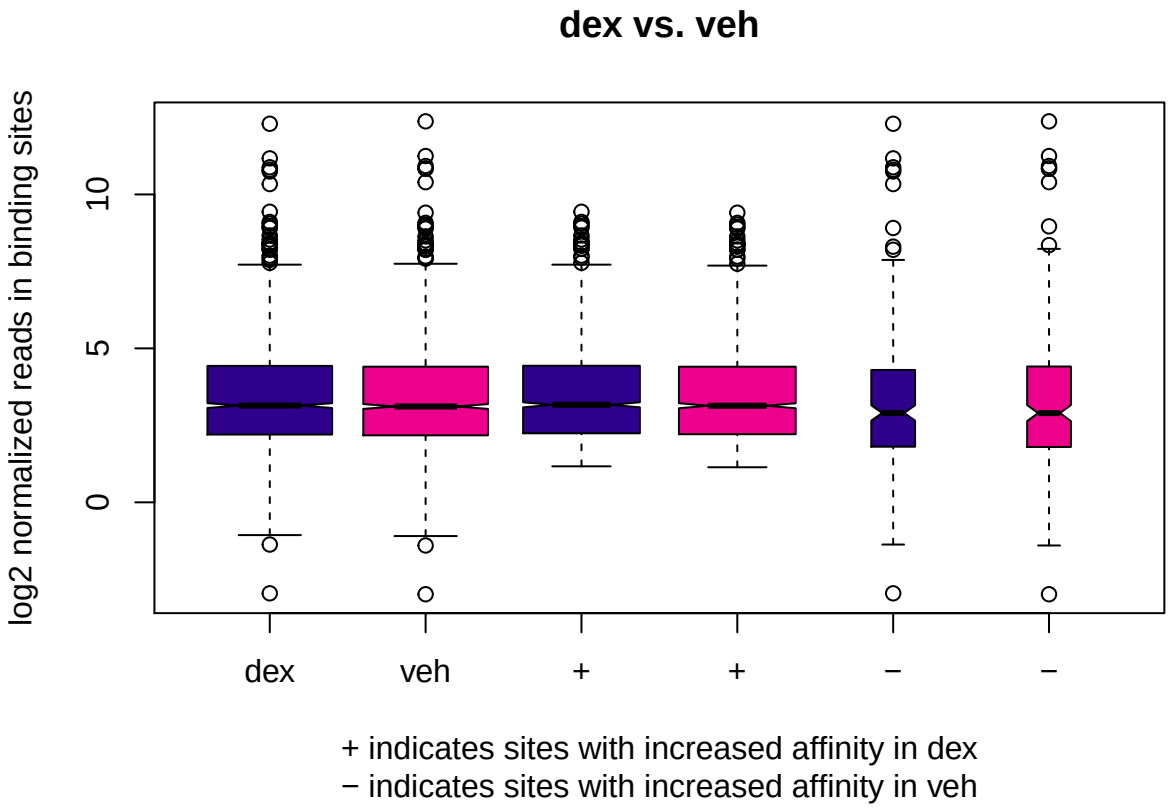
13.2. Volcano

```
dba.plotVolcano(dba.dif.anal.deseq, method = DBA_DESEQ2, contrast = 2)
```



13.3. Boxplot

```
dba.plotBox(dba.dif.anal.deseq, method = DBA_DESEQ2, contrast = 2)
```



+ indicates sites with increased affinity in dex
− indicates sites with increased affinity in veh