

Allele-specific ChIPseq analysis

Anastasiia Hryhorzhevskia

- 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/signer_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 MIPSYKL_000637    NA   ATAC      low      veh         1
## 2 MIPSYKL_002418    NA   ATAC      low      veh         1
## 3 MIPSYKL_003787    NA   ATAC     high     dex         2
## 4 MIPSYKL_001701    NA   ATAC     high     veh         1
## 5 MIPSYKL_003231    NA   ATAC     high     veh         1
##
## 1 /Users/anastasiia_hry/Documents/MPIP/Signe/08_peaks/07_filter_CNV/mpg_L18015_7C_veh_S26.cutadapt.
## 2 /Users/anastasiia_hry/Documents/MPIP/Signe/08_peaks/07_filter_CNV/mpg_L18015_14D_veh_S33.cutadapt.
## 3 /Users/anastasiia_hry/Documents/MPIP/Signe/08_peaks/07_filter_CNV/mpg_L18015_15B_dex_S72.cutadapt.
## 4 /Users/anastasiia_hry/Documents/MPIP/Signe/08_peaks/07_filter_CNV/mpg_L18015_14B_veh_S60.cutadapt.
## 5 /Users/anastasiia_hry/Documents/MPIP/Signe/08_peaks/07_filter_CNV/mpg_L18015_18D_veh_S62.cutadapt.
##
## 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)
```

```
## MIPSYKL_000637  ATAC low veh 1 macs
## MIPSYKL_002418  ATAC low veh 1 macs
## MIPSYKL_003787  ATAC high dex 2 macs
## MIPSYKL_001701  ATAC high veh 1 macs
## MIPSYKL_003231  ATAC high veh 1 macs
## MIPSYKL_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)
```

```
## [1] "/Users/anastasiia_hry/github/mpip/signe_chip-seq/03_result//dba_Signe_ChIPseq.RData"
```

```

# 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$mask$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$mask$dex & dba.db$mask$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$mask$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$mask$veh & dba.db$mask$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):
```

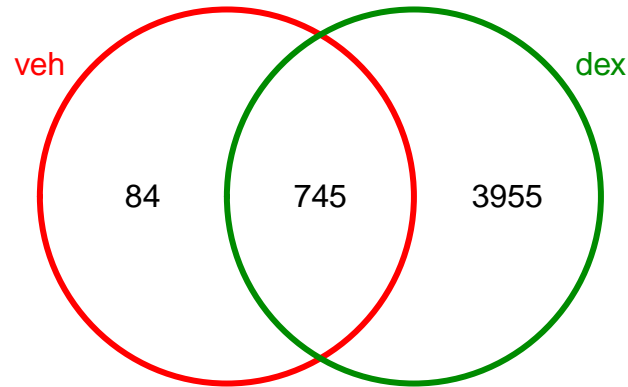
```
## ID Factor Condition Treatment Replicate Intervals
## 1 MPIPSYKL_000637 ATAC low veh 1 460
## 2 MPIPSYKL_002418 ATAC low veh 1 354
## 3 MPIPSYKL_003787 ATAC high dex 2 1955
```

## 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$mask$Consensus)
```

Binding Site Overlaps



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$mask$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.

```
# 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...
```

```
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 differentially 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 MIPISYKL_002319 ATAC low dex 2 56561.0 0.58
## 92 MIPISYKL_003785 ATAC high veh 1 217473.5 0.29
## 93 MIPISYKL_001048 ATAC high dex 2 101095.5 0.60
## 94 MIPISYKL_002095 ATAC low dex 2 809162.0 0.36
## 95 MIPISYKL_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 :

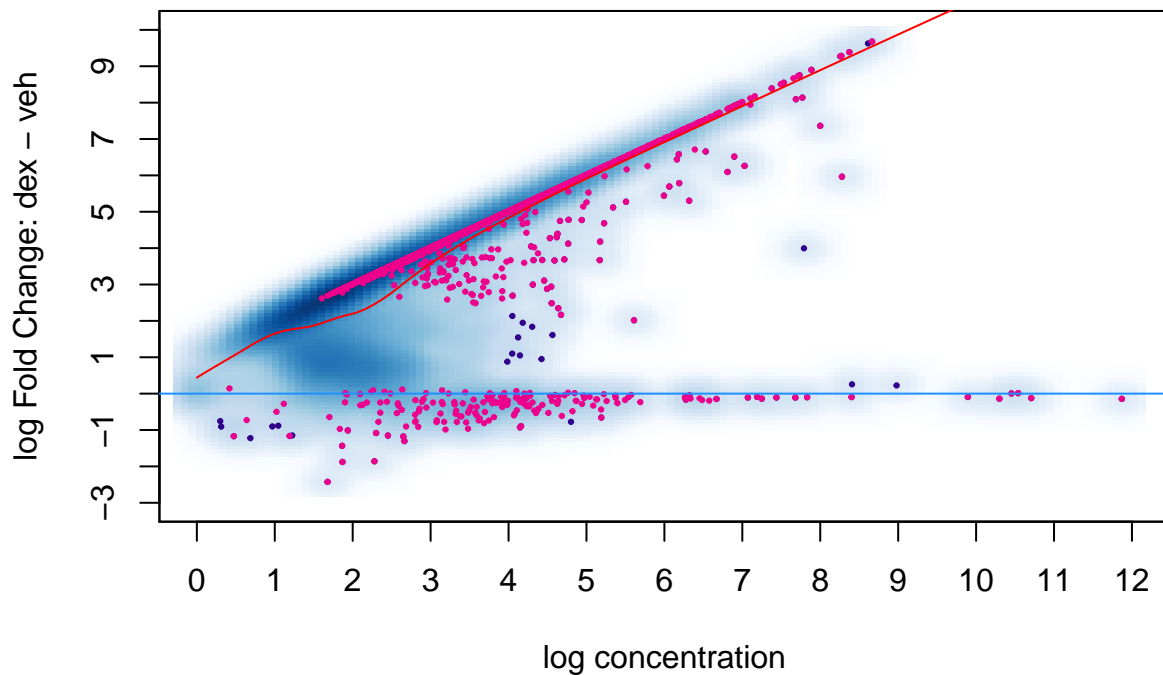
FDR = 5% :

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

EdgeR : 3285 of the 4781

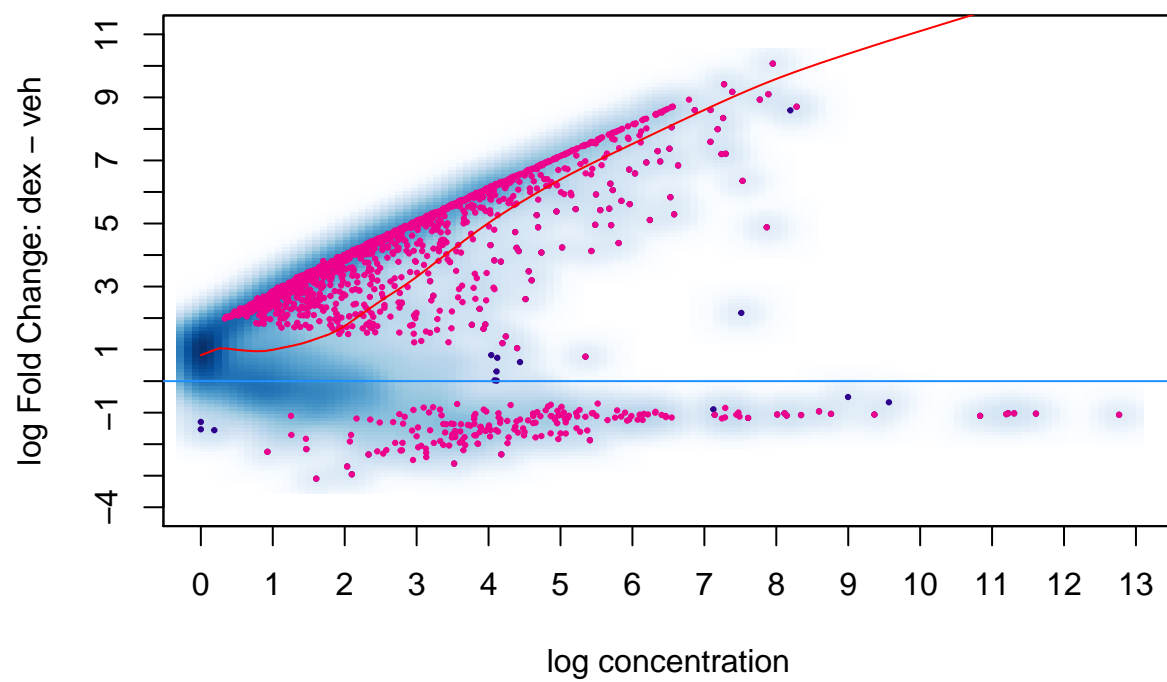
```
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2, bNormalized=F)
```

dex vs. veh (1809 FDR < 0.050)



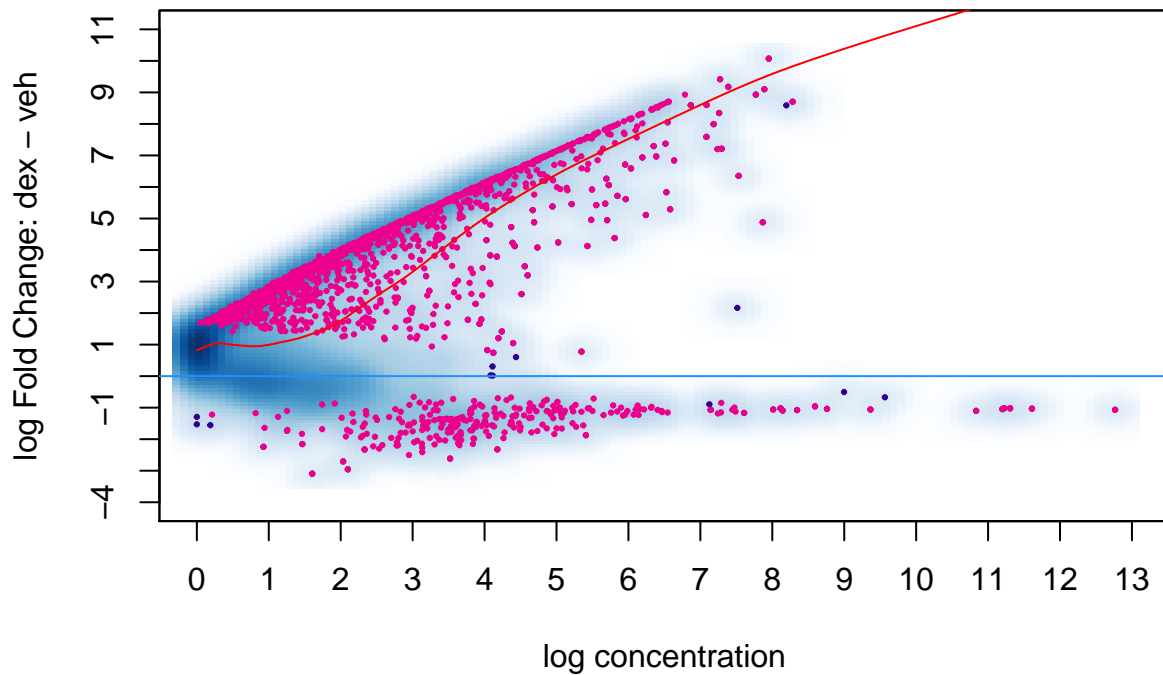
```
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2)
```

dex vs. veh (1809 FDR < 0.050)



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

dex vs. veh (2082 FDR < 0.100)



8.2. In-built normalization

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

```
## Applying Blacklist/Greylis...
```

```
## Genome detected: Hsapiens.NCBI.GRCh38
```

```
## Applying blacklist...
```

```
## Removed: 3 of 4784 intervals.
```

```
## No control reads specified, unable to generate greylis.
```

```
## 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:

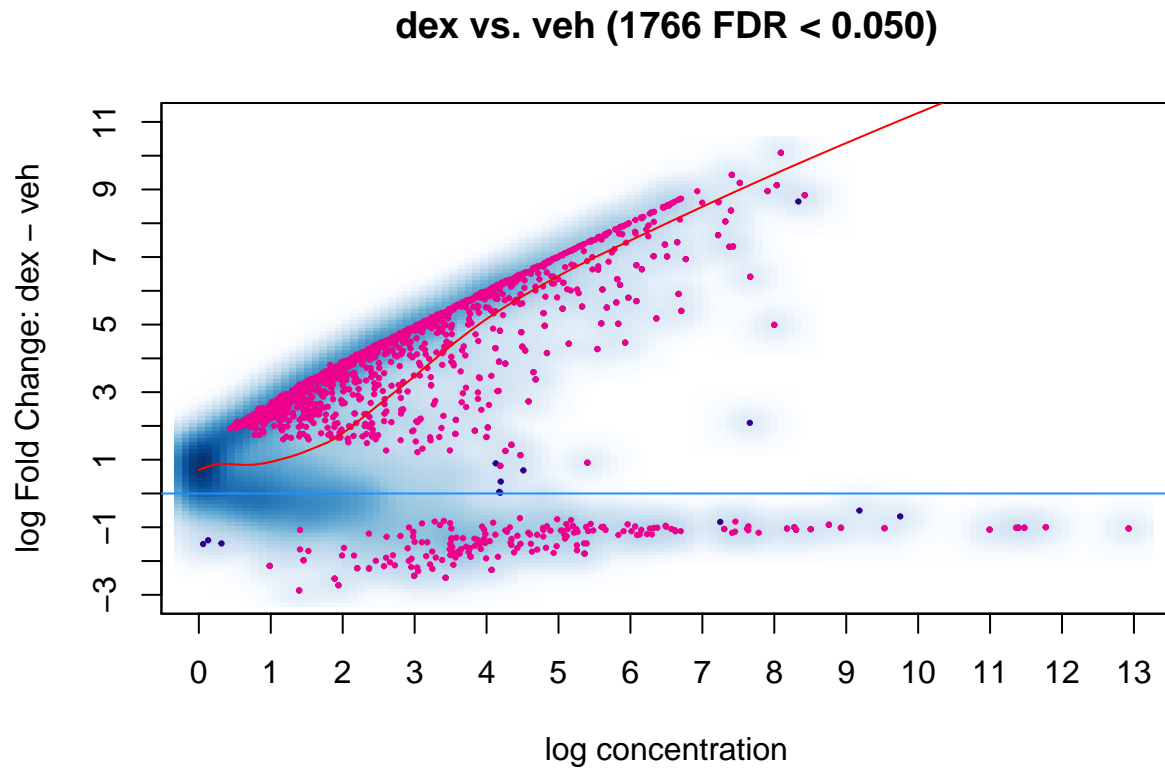
##	Factor	Group	Samples	Group2	Samples2	DB.edgeR	DB.DESeq2
## 1	Condition	low	47	high	48	4	0
## 2	Treatment	dex	47	veh	48	3270	1766

Result :

FDR = 5% :

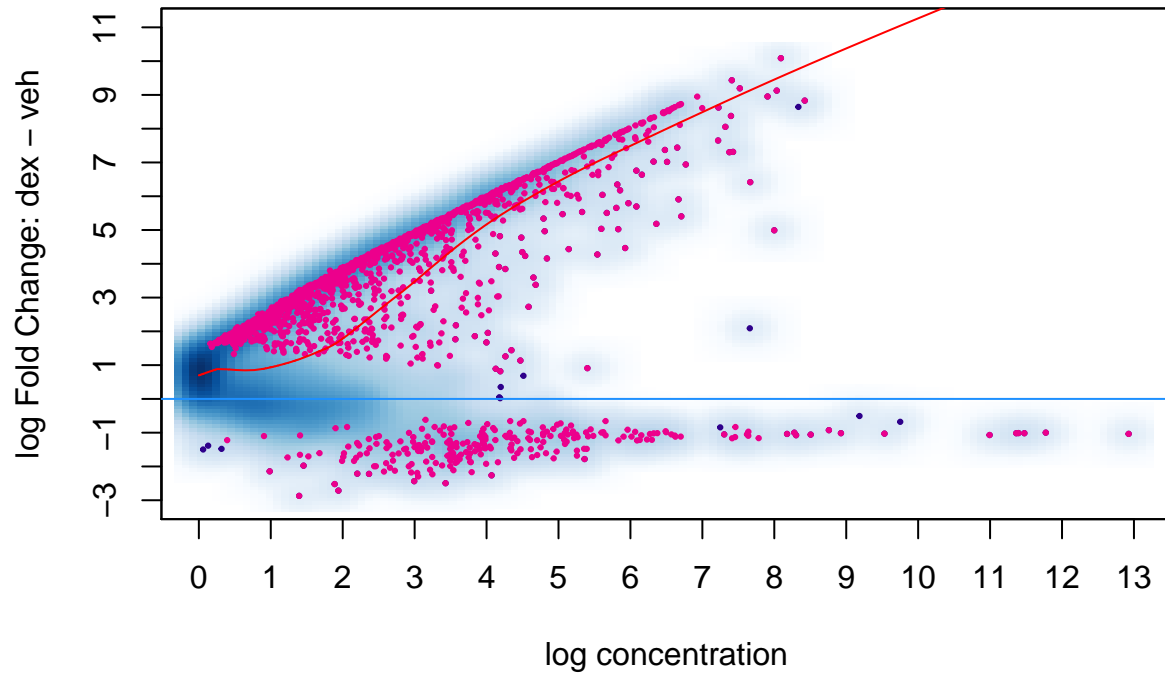
DeSeq : 1766 of the 4781 are identified as being significantly differentially bound
EdgeR : 3270 of the 4781

```
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)
```

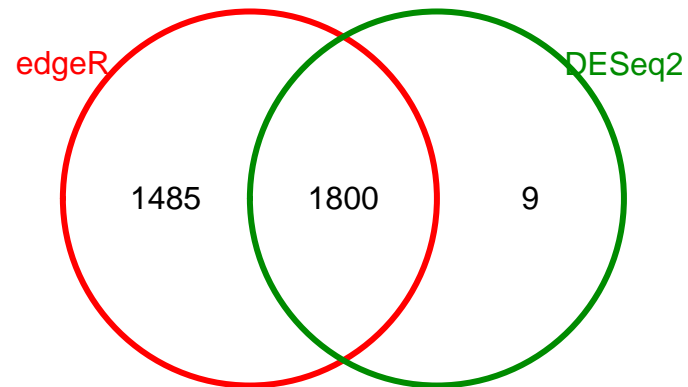

dex vs. veh (2035 FDR < 0.100)



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

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

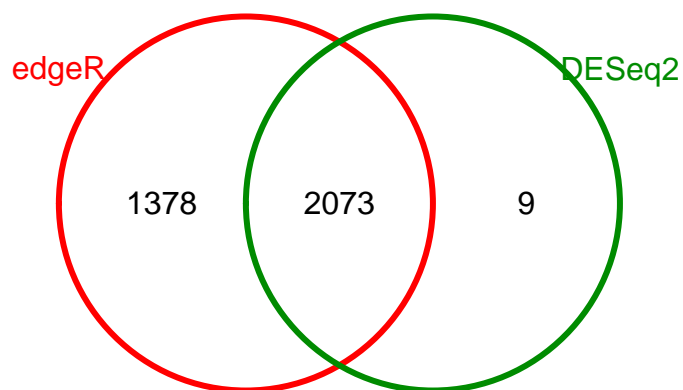
Binding Site Overlaps, FDR = 5%



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 =
```

Binding Site Overlaps, FDR = 10%



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$config$th,
#                                     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 dif bound sites

```
dex.report <- dba.report(dba.dif.anal.deseq, contrast = 2, method = DBA_DESEQ2, th = dba.dif.anal.deseq$config$th,
                        bCounts = T, bCalled = T) # file = paste0(result.dir.fn, "dex_deseq_report.csv")
write.csv2(dex.report, file = 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
##      MIPSYKL_003155 MIPSYKL_001886 MIPSYKL_003787 MIPSYKL_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
##      MIPSYKL_002852 MIPSYKL_002613 MIPSYKL_003911 MIPSYKL_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
##      MIPSYKL_002533 MIPSYKL_001547 MIPSYKL_004100 MIPSYKL_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
##      MIPSYKL_003162 MIPSYKL_002658 MIPSYKL_004198 MIPSYKL_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
##      MIPSYKL_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
```

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 values
```

```
## stop_codon. This information was ignored.
```

```
## OK
```

```
# annotate all peaks
```

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

```
## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the other
```

```
## - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI270312.1
```

```
## - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI270728.1
```

```
## 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 other
```

```
## - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI270312.1
```

```
## - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI270728.1
```

```
## 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 other
```

```
## - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI270312.1
```

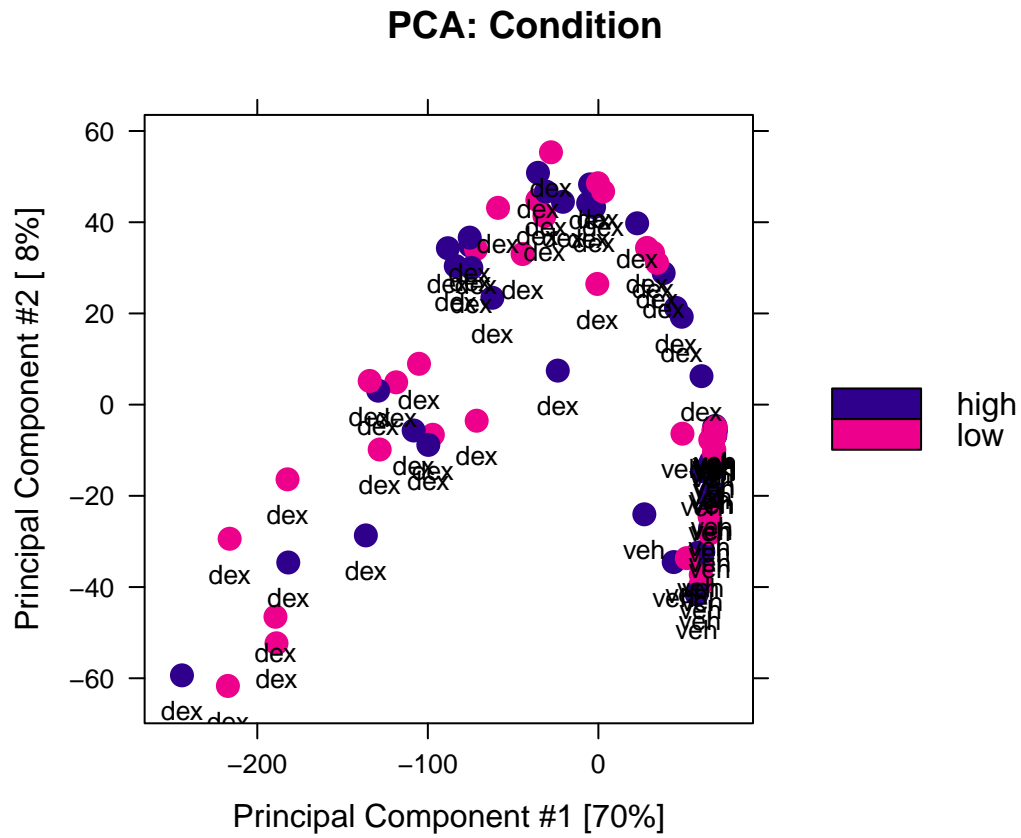
```
## - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI270728.1
```

```
## Make sure to always combine/compare objects based on the same reference
```

```
## genome (use suppressWarnings() to suppress this warning).
```

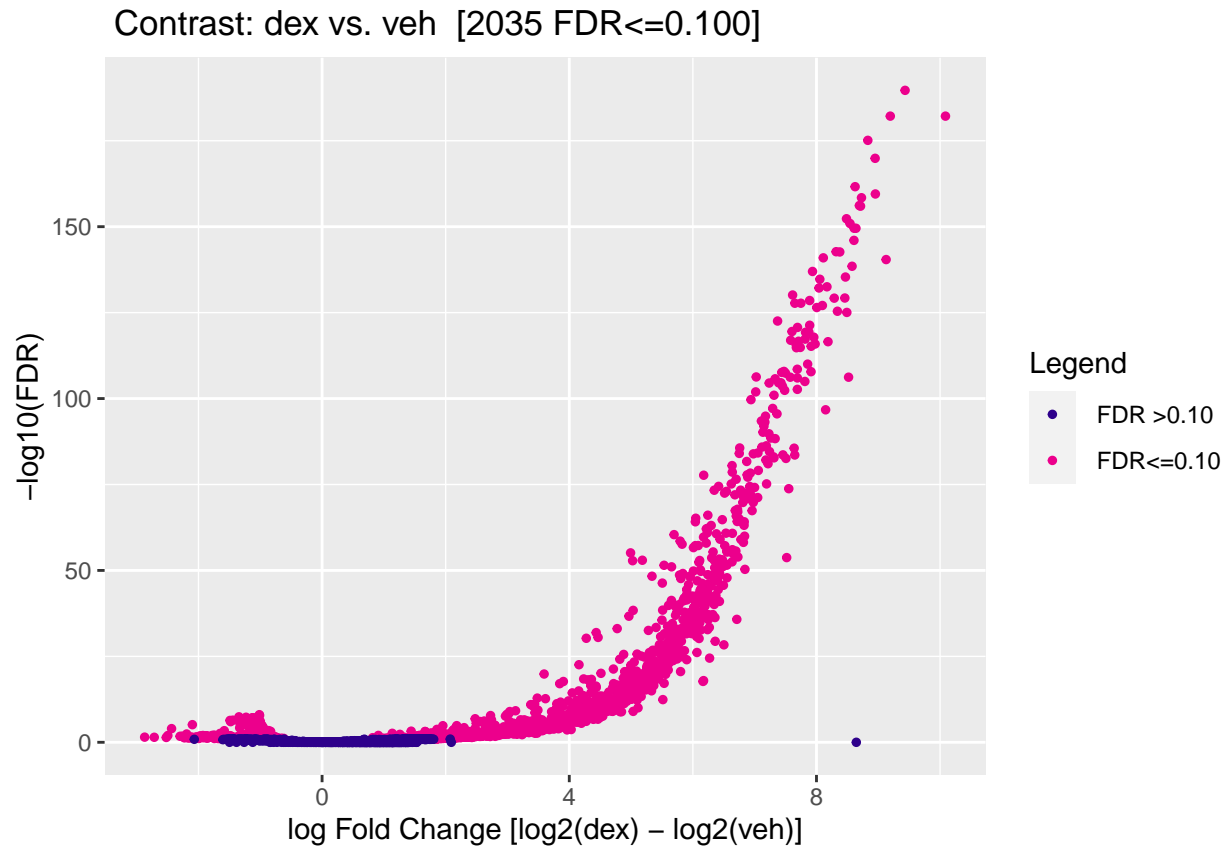


```
dba.plotPCA(dba.dif.anal.deseq, method = DBA_DESEQ2, attributes = DBA_CONDITION, label = DBA_TREATMENT,
```



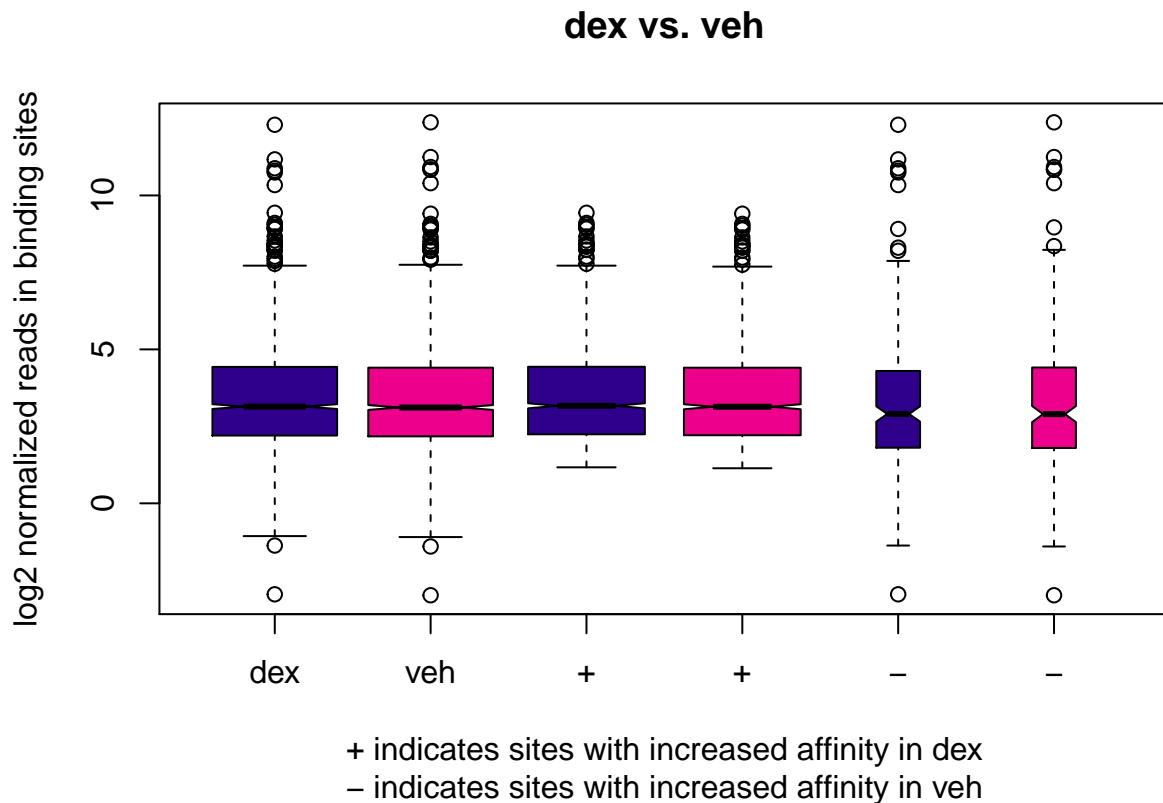
13.2. Volcano

```
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)
```



14. Export only dex enriched results

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

```
dex.enrich.df <- annotation.df %>% filter(FDR < 0.1 & Fold > 0)

write.csv2(dex.enrich.df, file = paste0(result.dir.fn, "dex_deseq_report_annotated_only_dex_enriched.csv"))

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

# veh.enrich <- out.edger %>%
#   filter(FDR < 0.05 & Fold < 0) %>%
#   select(seqnames, start, end)
#
# write.table(veh.enrich, file = paste0(result.dir.fn, "veh_enriched.bed"), sep = "\t", quote = F, row.names = F)
```