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/"</pre>
setwd(wd)
result.dir.fn <- paste0(wd, "03_result/")
sample.sheet.fn <- paste0(wd, "01_sample_sheets/chipseq_diffbind_sample_sheet_local.csv")</pre>
samples <- read.csv2(sample.sheet.fn, head = T)[, -11]</pre>
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
                                                                 2
                         NA
                              ATAC
                                         high
                                                    dex
## 4 MPIPSYKL_001701
                         NA
                              ATAC
                                         high
                                                    veh
                                                                 1
## 5 MPIPSYKL_003231
                         NA
                              ATAC
                                         high
                                                    veh
##
## 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)</pre>
## 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 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(pasteO(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
                                                                460
                                            veh
                                                        1
## 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
                                                        1
                                                                236
                                 high
                                            veh
## 6 MPIPSYKL 004171
                                                               2699
                       ATAC
                                  low
                                            dex
```

MPIPSYKL_001386 ATAC high veh 1 macs

##	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
##		MPIPSYKL_004215	ATAC	high	dex	2	2356
##		MPIPSYKL_001946	ATAC	high	dex	2	17256
##		MPIPSYKL_003353	ATAC	low	veh	1	3097
##		MPIPSYKL_002319	ATAC	low	veh	1	496
##		MPIPSYKL_001109	ATAC	high	veh	1	2130
##		MPIPSYKL_004251	ATAC	high	veh	1	410
##		MPIPSYKL_000708	ATAC	low	veh	1	760
##		MPIPSYKL_004251	ATAC	high	dex	2	563
##		MPIPSYKL_002802	ATAC	low	dex	2	1617
##		MPIPSYKL_002095	ATAC	low	veh	1	237
##		MPIPSYKL_003162	ATAC	low	dex	2	787
##		MPIPSYKL_002468	ATAC	high	veh	1	247 4077
##		MPIPSYKL_002658	ATAC	low	dex	2	4977
##		MPIPSYKL_003472	ATAC	high	veh	1 1	195 3423
##		MPIPSYKL_004054	ATAC	low	veh	2	3423 875
##		MPIPSYKL_002613	ATAC	high	dex	2	2073
##		MPIPSYKL_003231 MPIPSYKL_002533	ATAC ATAC	high high	dex dex	2	1120
##		MPIPSYKL_002802	ATAC	low	veh	1	838
##		MPIPSYKL_003934	ATAC	high	veh	1	259
##		MPIPSYKL_002418	ATAC	low	dex	2	708
		MPIPSYKL_001109	ATAC	high	dex	2	620
		MPIPSYKL_001786	ATAC	high	dex	2	858
		MPIPSYKL_003934	ATAC	high	dex	2	7158
		MPIPSYKL_002383	ATAC	low	veh	1	492
		MPIPSYKL_004215	ATAC	high	veh	1	400
		MPIPSYKL_004246	ATAC	high	dex	2	1145
		MPIPSYKL_001113	ATAC	high	veh	1	242
##		MPIPSYKL_001547	ATAC	high	dex	2	253
##		MPIPSYKL_001667	ATAC	high	veh	1	190
##		MPIPSYKL_000637	ATAC	low	dex	2	1141
##		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	${\tt high}$	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)
```

```
3458
    [1] 37757 21482 16679 13616 11128
                                                 7674
                                                               5523
                                                                      4707
                                                                            4062
                                           9285
                                                        6495
   [13]
          3071
                2731
                       2428
                              2180
                                    1986
                                           1832
                                                  1671
                                                        1519
                                                               1401
                                                                      1307
                                                                            1238
                                                                                   1156
   [25]
                        932
                               890
                                     838
                                                                632
                                                                       577
                                                                             541
                                                                                    500
          1085
                1016
                                            774
                                                   719
                                                         683
## [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
                                      3870
                                             2981
                                                    2350
                                                           1946
                                                                  1644
                                                                         1408
                                                                                1241
                        7477
                               5285
                                                                                       1112
## [13]
                                                                                 150
           973
                  845
                         747
                                665
                                       582
                                              496
                                                     407
                                                            328
                                                                   263
                                                                          206
                                                                                         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]
                                               127
                                                      124
                                                                                  107
                                                                                         101
           163
                  153
                         143
                                 139
                                       131
                                                             120
                                                                    114
                                                                           111
## [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)
```

```
178
    [1] 17310
                 6567
                        3583
                               2090
                                               751
                                                      531
                                                             397
                                                                    317
                                                                           262
                                                                                  212
                                      1219
## [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

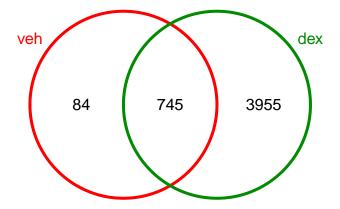
```
## 97 Samples, 24124 sites in matrix (44967 total):
##
                   ID Factor Condition Treatment Replicate Intervals
## 1 MPIPSYKL_000637
                         ATAC
                                    low
                                               veh
                              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

##	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
##		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
##		MPIPSYKL_000708	ATAC	low	veh	1	760
##		MPIPSYKL_004251	ATAC	high	dex	2	563
##		MPIPSYKL_002802	ATAC	low	dex	2	1617
##		MPIPSYKL_002095	ATAC	low	veh	1	237
##		MPIPSYKL_003162	ATAC	low	dex	2	787
##		MPIPSYKL_002468	ATAC	high	veh	1	247
##		MPIPSYKL_002658	ATAC	low	dex	2	4977
##		MPIPSYKL_003472	ATAC	high	veh	1	195
##		MPIPSYKL_004054	ATAC	low	veh	1	3423
##		MPIPSYKL_002613	ATAC	high	dex	2	875
##		MPIPSYKL_003231	ATAC	high	dex	2 2	2073
##		MPIPSYKL_002533	ATAC	high	dex	1	1120
		MPIPSYKL_002802	ATAC	low	veh		838
		MPIPSYKL_003934 MPIPSYKL_002418	ATAC ATAC	high low	veh	1 2	259 708
		MPIPSYKL_001109	ATAC		dex dex	2	620
		MPIPSYKL_001786	ATAC	high high	dex	2	858
		MPIPSYKL_003934	ATAC	high	dex	2	7158
		MPIPSYKL_002383	ATAC	low	veh	1	492
		MPIPSYKL_004215	ATAC	high	veh	1	400
		MPIPSYKL_004246	ATAC	high	dex	2	1145
		MPIPSYKL_001113	ATAC	high	veh	1	242
##		MPIPSYKL_001547	ATAC	high	dex	2	253
##		MPIPSYKL_001667	ATAC	high	veh	1	190
##		MPIPSYKL_000637	ATAC	low	dex	2	1141
		MPIPSYKL_001386	ATAC	high	dex	2	785
		MPIPSYKL_002468	ATAC	high	dex	2	1601
		MPIPSYKL_002088	ATAC	low	dex	2	4266
##		MPIPSYKL_003013	ATAC	high	veh	1	2791
##	57	MPIPSYKL_000972	ATAC	low	dex	2	3099

		MPIPSYKL_001048	ATAC	high	veh	1	3022
		MPIPSYKL_001389	ATAC	low	veh	1	635
		MPIPSYKL_003785	ATAC	high	dex	2	9905
		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
##		MPIPSYKL_002095	ATAC	low	dex	2	19071
##		MPIPSYKL_002088	ATAC	low	veh	1	3154
##	96	veh	ATAC	low-high	veh	1	832
##	97	dex	ATAC	high-low	dex	2	4707
## ## ## ## ## ##	89 90 91 92 93 94 95 96	MPIPSYKL_003353 MPIPSYKL_001667 MPIPSYKL_002319 MPIPSYKL_003785 MPIPSYKL_001048 MPIPSYKL_002095 MPIPSYKL_002088 veh	ATAC ATAC ATAC ATAC ATAC ATAC ATAC	low high low high high low low	dex dex veh dex veh veh	2 2 2 1 2 2 1 1	11524 5503 831 6017 2068 19071 3154 832

 ${\tt dba.plotVenn}({\tt dba.consensus}, \ {\tt dba.consensus} \$ {\tt masks} \$ {\tt Consensus})$

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\$masks\$Consensus, minOverlap = 1 dba.consensus.merge

##	98	Samples,	24124	sites in matri	(44967 to	tal):		
##			ID	Factor	Condition	Treatment	Replicate	${\tt Intervals}$
##	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

шш	40	MDIDGWWI 000070	A TT A CT	1	1-	4	F04
		MPIPSYKL_000972	ATAC	low	veh	1 2	581
##		MPIPSYKL_004100	ATAC	low	dex		19759
##		MPIPSYKL_003331	ATAC	low	veh	1	3347
##		MPIPSYKL_003520	ATAC	high	veh	1	192
##		MPIPSYKL_000988	ATAC	high	veh	1	1493
##		MPIPSYKL_004171	ATAC	low	veh	1	346
##		MPIPSYKL_003331	ATAC	low	dex	2	1706
##		MPIPSYKL_001766	ATAC	low	veh	1	136
##		MPIPSYKL_003520	ATAC	high	dex	2	2950
##		MPIPSYKL_004215	ATAC	high	dex	2	2356
##		MPIPSYKL_001946	ATAC	high	dex	2	17256
##		MPIPSYKL_003353	ATAC	low	veh	1	3097
##		MPIPSYKL_002319	ATAC	low	veh	1	496
##		MPIPSYKL_001109	ATAC	high	veh	1	2130
##		MPIPSYKL_004251	ATAC	high	veh	1	410
##		MPIPSYKL_000708	ATAC	low	veh	1	760
##		MPIPSYKL_004251	ATAC	high	dex	2	563
		MPIPSYKL_002802	ATAC	low	dex	2	1617
		MPIPSYKL_002095	ATAC	low	veh	1	237
		MPIPSYKL_003162	ATAC	low	dex	2	787
		MPIPSYKL_002468	ATAC	high	veh	1	247
		MPIPSYKL_002658	ATAC	low	dex	2	4977
		MPIPSYKL_003472	ATAC	high	veh	1	195
		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
##		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
                                                                       2
                                                                               2622
                                               low
                                                          dex
                                                                       2
## 68 MPIPSYKL_003155
                          ATAC
                                                                               1290
                                               low
                                                          dex
## 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
                                                                       2
                          ATAC
                                              high
                                                          dex
                                                                               1467
## 73 MPIPSYKL 002604
                          ATAC
                                               low
                                                                       1
                                                                                205
                                                          veh
## 74 MPIPSYKL_002533
                          ATAC
                                              high
                                                          veh
                                                                       1
                                                                                302
## 75 MPIPSYKL_001547
                          ATAC
                                              high
                                                                       1
                                                                                142
                                                          veh
                                                                       2
## 76 MPIPSYKL_000988
                          ATAC
                                              high
                                                          dex
                                                                               4918
## 77 MPIPSYKL_004100
                          ATAC
                                               low
                                                                       1
                                                                                598
                                                          veh
                                                                       2
## 78 MPIPSYKL_003472
                                                                                375
                          ATAC
                                              high
                                                          dex
## 79 MPIPSYKL_001386
                          ATAC
                                                                       1
                                                                                235
                                              high
                                                          veh
                                                                       2
## 80 MPIPSYKL_003911
                          ATAC
                                               low
                                                          dex
                                                                                470
## 81 MPIPSYKL_003162
                          ATAC
                                               low
                                                                       1
                                                                                219
                                                          veh
## 82 MPIPSYKL_001701
                          ATAC
                                                                       2
                                                                               3480
                                              high
                                                          dex
                                                                       2
## 83 MPIPSYKL_002383
                          ATAC
                                                                                836
                                               low
                                                          dex
## 84 MPIPSYKL 002658
                          ATAC
                                               low
                                                                       1
                                                          veh
                                                                                181
## 85 MPIPSYKL_001766
                                                                       2
                          ATAC
                                                                              11025
                                               low
                                                          dex
                                                                       2
## 86 MPIPSYKL_002852
                          ATAC
                                               low
                                                          dex
                                                                               8121
## 87 MPIPSYKL_001263
                          ATAC
                                              high
                                                                       2
                                                                               2089
                                                          dex
## 88 MPIPSYKL_004198
                          ATAC
                                               low
                                                          veh
                                                                       1
                                                                                181
                                                                       2
## 89 MPIPSYKL_003353
                          ATAC
                                               low
                                                          dex
                                                                              11524
                                                                       2
## 90 MPIPSYKL_001667
                          ATAC
                                              high
                                                          dex
                                                                               5503
                                                                       2
## 91 MPIPSYKL_002319
                          ATAC
                                               low
                                                          dex
                                                                                831
## 92 MPIPSYKL_003785
                          ATAC
                                              high
                                                          veh
                                                                       1
                                                                               6017
## 93 MPIPSYKL_001048
                                                                       2
                                                                               2068
                          ATAC
                                              high
                                                          dex
                                                                       2
## 94 MPIPSYKL_002095
                          ATAC
                                               low
                                                                              19071
                                                          dex
## 95 MPIPSYKL_002088
                                                                       1
                          ATAC
                                               low
                                                          veh
                                                                               3154
## 96
                          ATAC
                                                                                832
                   veh
                                         low-high
                                                          veh
                                                                       1
## 97
                   dex
                          ATAC
                                         high-low
                                                          dex
                                                                       2
                                                                               4707
## 98
                          ATAC low-high-high-low
                                                                     1-2
                                                                               4784
               veh-dex
                                                      veh-dex
```

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</pre>
```

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)</pre>
```

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(pasteO(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:			
##		-	I	D Factor	Condition	Treatment	Replicate	Reads FRiP
##	1	MPIPSYKL_	_00063	7 ATAC	low	veh	1	48646.5 0.48
##	2	MPIPSYKL_	00241	8 ATAC	low	veh	1	39876.0 0.41
##	3	MPIPSYKL_	_00378	7 ATAC	high	dex	2	122369.5 0.60
##	4	MPIPSYKL_	_00170	1 ATAC	high	veh	1	252860.5 0.28
##	5	MPIPSYKL_	_00323	1 ATAC	high	veh	1	32347.0 0.38
##	6	MPIPSYKL_	_00417	1 ATAC	low	dex	2	173266.5 0.61
##	7	MPIPSYKL_	_00260	4 ATAC	low	dex	2	73592.0 0.63
##	8	MPIPSYKL_	_00405	4 ATAC	low	dex	2	133546.5 0.65
##	9	MPIPSYKL_			high	veh	1	25906.5 0.46
##		MPIPSYKL_	_		high	veh	1	38769.0 0.35
##		MPIPSYKL_			high	veh	1	44678.0 0.41
##		MPIPSYKL_			high	dex	2	26133.0 0.45
##		MPIPSYKL_	-		low	veh	1	44139.0 0.42
##		MPIPSYKL_	-		low	dex	2	1093528.0 0.38
##		MPIPSYKL_			low	veh	1	136884.0 0.33
##		MPIPSYKL_	_		high	veh	1	34792.5 0.37
##		MPIPSYKL_			high	veh	1	97502.5 0.35
##		MPIPSYKL_	_		low	veh	1	59254.5 0.34
##		MPIPSYKL_	_		low	dex	2	80213.0 0.70
##		MPIPSYKL_	_		low	veh	1	29695.5 0.35
##		MPIPSYKL_			high	dex	2	137986.5 0.72
##		MPIPSYKL_			high	dex	2	120145.0 0.63
##		MPIPSYKL_			high	dex		1128909.5 0.51
##		MPIPSYKL_	_		low	veh	1	136983.5 0.34
##		MPIPSYKL_			low	veh	1	50702.5 0.51
##		MPIPSYKL_	_		high	veh	1	89583.0 0.32
##		MPIPSYKL_			high	veh	1	35730.5 0.42
##		MPIPSYKL_	_		low	veh	1	33399.5 0.39
##		MPIPSYKL_	_		high	dex	2	44901.0 0.41
##		MPIPSYKL_	_		low	dex	2	79331.0 0.60
## ##		MPIPSYKL_	_		low low	veh dex	1 2	32884.0 0.44 41535.5 0.51
##		MPIPSYKL_MPIPSYKL_			high	veh	1	38420.0 0.42
##		MPIPSYKL			low	dex	2	315901.5 0.67
		MPIPSYKL_			high	veh	1	26032.0 0.47
					low	ven	1	174999.5 0.32
		MPIPSYKL_MPIPSYKL			high	dex	2	80250.5 0.49
		MPIPSYKL	-		high	dex	2	88498.0 0.65
		MPIPSYKL	_		high	dex	2	113871.0 0.45
		MPIPSYKL	_		low	veh	1	52387.5 0.35
##		MPIPSYKL_	_		high	veh	1	32213.5 0.39
##		MPIPSYKL			low	dex	2	56650.5 0.53
##		MPIPSYKL_			high	dex	2	45694.0 0.56
		MPIPSYKL			high	dex	2	65412.0 0.55
ıτπ		''' TI DIIVE	_001/0	O AIAO	111 EII	uex	2	00412.0 0.00

```
## 45 MPIPSYKL 003934
                          ATAC
                                                                 357234.0 0.60
                                     high
                                                 dex
## 46 MPIPSYKL_002383
                          ATAC
                                      low
                                                              1
                                                                  38184.5 0.35
                                                 veh
## 47 MPIPSYKL 004215
                          ATAC
                                     high
                                                 veh
                                                              1
                                                                  31424.0 0.48
## 48 MPIPSYKL_004246
                          ATAC
                                                              2
                                     high
                                                 dex
                                                                  86453.5 0.62
## 49 MPIPSYKL_001113
                          ATAC
                                     high
                                                 veh
                                                              1
                                                                  36781.5 0.35
                                                              2
                                                                  20935.0 0.57
## 50 MPIPSYKL 001547
                                    high
                          ATAC
                                                 dex
## 51 MPIPSYKL 001667
                          ATAC
                                     high
                                                 veh
                                                              1
                                                                  33561.5 0.38
## 52 MPIPSYKL_000637
                                                              2
                          ATAC
                                      low
                                                 dex
                                                                  84438.0 0.62
## 53 MPIPSYKL_001386
                          ATAC
                                    high
                                                 dex
                                                              2
                                                                  52700.0 0.59
                                                              2
## 54 MPIPSYKL_002468
                          ATAC
                                    high
                                                 dex
                                                                  92503.5 0.71
## 55 MPIPSYKL_002088
                          ATAC
                                      low
                                                              2
                                                                 178600.5 0.63
                                                 dex
## 56 MPIPSYKL_003013
                          ATAC
                                     high
                                                 veh
                                                              1
                                                                 101447.5 0.35
  57 MPIPSYKL_000972
                          ATAC
                                                              2
                                                                 112234.5 0.43
                                      low
                                                 dex
## 58 MPIPSYKL_001048
                                                                  95489.5 0.27
                          ATAC
                                     high
                                                 veh
                                                              1
## 59 MPIPSYKL_001389
                          ATAC
                                      low
                                                 veh
                                                              1
                                                                  50559.5 0.44
## 60 MPIPSYKL_003785
                          ATAC
                                     high
                                                              2
                                                                 378592.5 0.43
                                                 dex
## 61 MPIPSYKL_003155
                                      low
                                                              1
                          ATAC
                                                 veh
                                                                  41054.5 0.36
## 62 MPIPSYKL 001886
                          ATAC
                                      low
                                                              1
                                                                  98282.5 0.37
                                                 veh
## 63 MPIPSYKL_003787
                          ATAC
                                    high
                                                              1
                                                                  27634.0 0.45
                                                 veh
## 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
                                                              1
                                                                 199912.5 0.31
                          ATAC
                                     high
                                                 veh
                                                              2
## 67 MPIPSYKL_001389
                          ATAC
                                      low
                                                                 157832.0 0.74
                                                 dex
                                                              2
## 68 MPIPSYKL 003155
                          ATAC
                                      low
                                                 dex
                                                                  77820.0 0.67
                                                              2
## 69 MPIPSYKL 000708
                          ATAC
                                      low
                                                 dex
                                                                  96744.0 0.57
  70 MPIPSYKL_003911
                          ATAC
                                      low
                                                 veh
                                                              1
                                                                  18954.5 0.55
                                                              2
                                                                 208394.5 0.48
  71 MPIPSYKL_001886
                          ATAC
                                      low
                                                 dex
                                                              2
## 72 MPIPSYKL_003013
                          ATAC
                                    high
                                                                  62343.5 0.62
                                                 dex
                                                              1
## 73 MPIPSYKL_002604
                          ATAC
                                      low
                                                 veh
                                                                  25425.5 0.47
## 74 MPIPSYKL_002533
                          ATAC
                                                              1
                                                                  41036.0 0.30
                                     high
                                                 veh
## 75 MPIPSYKL_001547
                          ATAC
                                     high
                                                 veh
                                                              1
                                                                  27024.5 0.43
## 76 MPIPSYKL_000988
                          ATAC
                                    high
                                                              2
                                                                 200150.0 0.52
                                                 dex
## 77 MPIPSYKL_004100
                          ATAC
                                      low
                                                              1
                                                                  66633.5 0.39
                                                 veh
                                                              2
## 78 MPIPSYKL_003472
                          ATAC
                                                                  37347.5 0.45
                                    high
                                                 dex
  79 MPIPSYKL 001386
                          ATAC
                                                              1
                                                                  35902.5 0.42
                                     high
                                                 veh
                                                              2
## 80 MPIPSYKL_003911
                          ATAC
                                      low
                                                 dex
                                                                  30382.5 0.57
## 81 MPIPSYKL 003162
                          ATAC
                                      low
                                                 veh
                                                              1
                                                                  36747.0 0.39
## 82 MPIPSYKL_001701
                          ATAC
                                                              2
                                                                  95701.5 0.43
                                    high
                                                 dex
                                                              2
## 83 MPIPSYKL_002383
                          ATAC
                                      low
                                                 dex
                                                                  67016.0 0.46
                                                              1
                                                                  21971.5 0.48
## 84 MPIPSYKL_002658
                          ATAC
                                      low
                                                 veh
                                                              2
## 85 MPIPSYKL 001766
                          ATAC
                                      low
                                                 dex
                                                                 343078.0 0.33
## 86 MPIPSYKL 002852
                          ATAC
                                                              2
                                                                 576255.5 0.64
                                      low
                                                 dex
## 87 MPIPSYKL_001263
                          ATAC
                                    high
                                                 dex
                                                              2
                                                                 112898.5 0.69
                                                              1
  88 MPIPSYKL_004198
                          ATAC
                                      low
                                                 veh
                                                                  19719.5 0.53
                                                              2
## 89 MPIPSYKL_003353
                          ATAC
                                      low
                                                                 485290.5 0.52
                                                 dex
                                                              2
## 90 MPIPSYKL_001667
                          ATAC
                                     high
                                                 dex
                                                                 158705.5 0.47
                                                              2
## 91 MPIPSYKL_002319
                          ATAC
                                      low
                                                 dex
                                                                  56561.0 0.58
## 92 MPIPSYKL_003785
                          ATAC
                                     high
                                                 veh
                                                              1
                                                                 217473.5 0.29
  93 MPIPSYKL_001048
                          ATAC
                                     high
                                                              2
                                                                 101095.5 0.60
                                                 dex
   94 MPIPSYKL_002095
                          ATAC
                                      low
                                                                 809162.0 0.36
                                                 dex
##
   95 MPIPSYKL_002088
                                                                 106472.0 0.33
                          ATAC
                                      low
                                                 veh
## Design: [~Condition + Treatment] | 2 Contrasts:
```

16

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)</pre>
```

dba.dif.anal.norm

```
## 95 Samples, 4781 sites in matrix (4784 total):
                    ID Factor Condition Treatment Replicate
##
                                                                    Reads FRiP
##
      MPIPSYKL_000637
                          ATAC
                                      low
                                                veh
                                                                  48646.5 0.48
##
  2
      MPIPSYKL_002418
                          ATAC
                                     low
                                                             1
                                                                  39876.0 0.41
                                                veh
                                                             2
  3
      MPIPSYKL_003787
                          ATAC
                                                                 122369.5 0.60
                                    high
                                                dex
      MPIPSYKL_001701
                          ATAC
                                                                 252860.5 0.28
## 4
                                    high
                                                             1
                                                veh
                          ATAC
                                                             1
## 5
      MPIPSYKL_003231
                                    high
                                                veh
                                                                  32347.0 0.38
## 6
      MPIPSYKL_004171
                          ATAC
                                     low
                                                dex
                                                             2
                                                                 173266.5 0.61
      MPIPSYKL_002604
                          ATAC
                                     low
                                                dex
                                                             2
                                                                  73592.0 0.63
                                                             2
      MPIPSYKL_004054
                          ATAC
## 8
                                     low
                                                dex
                                                                 133546.5 0.65
## 9
      MPIPSYKL_001263
                          ATAC
                                    high
                                                veh
                                                             1
                                                                  25906.5 0.46
## 10 MPIPSYKL_001786
                         ATAC
                                    high
                                                             1
                                                                  38769.0 0.35
                                                veh
## 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
                                                             1
                                                                  44139.0 0.42
                                                veh
## 14 MPIPSYKL_004100
                          ATAC
                                     low
                                                dex
                                                             2 1093528.0 0.38
## 15 MPIPSYKL_003331
                         ATAC
                                                                 136884.0 0.33
                                     low
                                                             1
                                                veh
## 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
                                                             2
## 19 MPIPSYKL_003331
                          ATAC
                                     low
                                                                  80213.0 0.70
                                                dex
## 20 MPIPSYKL_001766
                          ATAC
                                                             1
                                                                  29695.5 0.35
                                     low
                                                veh
## 21 MPIPSYKL_003520
                                                             2
                         ATAC
                                    high
                                                dex
                                                                 137986.5 0.72
## 22 MPIPSYKL_004215
                          ATAC
                                    high
                                                dex
                                                                 120145.0 0.63
                                                             2
## 23 MPIPSYKL_001946
                          ATAC
                                    high
                                                dex
                                                               1128909.5 0.51
## 24 MPIPSYKL_003353
                          ATAC
                                     low
                                                veh
                                                             1
                                                                 136983.5 0.34
## 25 MPIPSYKL_002319
                         ATAC
                                     low
                                                             1
                                                                  50702.5 0.50
                                                veh
## 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
                                                             1
                                                                  33399.5 0.39
                                                veh
                                                             2
## 29 MPIPSYKL_004251
                          ATAC
                                    high
                                                dex
                                                                  44901.0 0.41
## 30 MPIPSYKL_002802
                          ATAC
                                     low
                                                             2
                                                                  79331.0 0.60
                                                dex
## 31 MPIPSYKL_002095
                          ATAC
                                     low
                                                veh
                                                             1
                                                                  32884.0 0.44
## 32 MPIPSYKL_003162
                         ATAC
                                                             2
                                     low
                                                dex
                                                                  41535.5 0.51
## 33 MPIPSYKL 002468
                          ATAC
                                    high
                                                             1
                                                                  38420.0 0.42
                                                veh
## 34 MPIPSYKL_002658
                                                             2
                                                                315901.5 0.67
                          ATAC
                                     low
                                                dex
## 35 MPIPSYKL_003472
                          ATAC
                                    high
                                                veh
                                                             1
                                                                  26032.0 0.47
## 36 MPIPSYKL_004054
                         ATAC
                                     low
                                                veh
                                                                174999.5 0.32
```

##	27	MPIPSYKL 002613	ATAC	h i mh	dex	2	80250.5 0.49
		MPIPSYKL_003231	ATAC	high high	dex	2	88498.0 0.65
		MPIPSYKL_003231	ATAC	high	dex	2	113871.0 0.45
		MPIPSYKL 002802	ATAC	low	veh	1	52387.5 0.35
		MPIPSYKL_003934	ATAC		ven	1	32213.5 0.39
		MPIPSYKL_003934	ATAC	high low	dex	2	56650.5 0.53
		_	ATAC			2	45694.0 0.56
		MPIPSYKL_001109	ATAC	high high	dex	2	65412.0 0.55
		MPIPSYKL_001786	ATAC	_	dex	2	357234.0 0.60
		MPIPSYKL_003934		high low	dex	1	38184.5 0.34
		MPIPSYKL_002383	ATAC		veh		31424.0 0.48
		MPIPSYKL_004215	ATAC	high	veh	1	
		MPIPSYKL_004246	ATAC	high	dex	2	86453.5 0.62
		MPIPSYKL_001113	ATAC	high	veh	1	36781.5 0.35
##		MPIPSYKL_001547	ATAC	high	dex	2	20935.0 0.57
##		MPIPSYKL_001667	ATAC	high	veh	1	33561.5 0.38
##		MPIPSYKL_000637	ATAC	low	dex	2	84438.0 0.62
##		MPIPSYKL_001386	ATAC	high	dex	2	52700.0 0.59
##		MPIPSYKL_002468	ATAC	high	dex	2	92503.5 0.71
##		MPIPSYKL_002088	ATAC	low	dex	2	178600.5 0.63
##		MPIPSYKL_003013	ATAC	high	veh	1	101447.5 0.35
##		MPIPSYKL_000972	ATAC	low	dex	2	112234.5 0.43
##		MPIPSYKL_001048	ATAC	high	veh	1	95489.5 0.27
##		MPIPSYKL_001389	ATAC	low	veh	1	50559.5 0.44
##		MPIPSYKL_003785	ATAC	high	dex	2	378592.5 0.43
##		MPIPSYKL_003155	ATAC	low	veh	1	41054.5 0.36
##		MPIPSYKL_001886	ATAC	low	veh	1	98282.5 0.37
##		MPIPSYKL_003787	ATAC	high	veh	1	27634.0 0.45
##		MPIPSYKL_004246	ATAC	high	veh	1	49954.0 0.53
##		MPIPSYKL_002852	ATAC	low	veh	1	32191.5 0.47
##		MPIPSYKL_002613	ATAC	high	veh	1	199912.5 0.31
##		MPIPSYKL_001389	ATAC	low	dex	2	157832.0 0.74
##		MPIPSYKL_003155	ATAC	low	dex	2	77820.0 0.67
##		MPIPSYKL_000708	ATAC	low	dex	2	96744.0 0.57
##		MPIPSYKL_003911	ATAC	low	veh	1	18954.5 0.55
##		MPIPSYKL_001886	ATAC	low	dex	2	208394.5 0.48
##		MPIPSYKL_003013	ATAC	high	dex	2	62343.5 0.62
		MPIPSYKL_002604	ATAC	low	veh	1	25425.5 0.47
		MPIPSYKL_002533	ATAC	high	veh	1	41036.0 0.30
		MPIPSYKL_001547	ATAC	high	veh	1	27024.5 0.43
		MPIPSYKL_000988	ATAC	high	dex	2	200150.0 0.52
		MPIPSYKL_004100	ATAC	low	veh	1	66633.5 0.39
		MPIPSYKL_003472	ATAC	high	dex	2	37347.5 0.45
		MPIPSYKL_001386	ATAC	high	veh	1	35902.5 0.42
		MPIPSYKL_003911	ATAC	low	dex	2	30382.5 0.57
		MPIPSYKL_003162	ATAC	low	veh	1	36747.0 0.39
		MPIPSYKL_001701	ATAC	high	dex	2	95701.5 0.43
		MPIPSYKL_002383	ATAC	low	dex	2	67016.0 0.46
##	84	MPIPSYKL_002658	ATAC	low	veh	1	21971.5 0.48
		MPIPSYKL_001766	ATAC	low	dex	2	343078.0 0.33
		MPIPSYKL_002852	ATAC	low	dex	2	576255.5 0.64
##	87	MPIPSYKL_001263	ATAC	high	dex	2	112898.5 0.69
		MPIPSYKL_004198	ATAC	low	veh	1	19719.5 0.53
		MPIPSYKL_003353	ATAC	low	dex	2	485290.5 0.52
##	90	MPIPSYKL_001667	ATAC	high	dex	2	158705.5 0.47

```
ATAC
## 91 MPIPSYKL_002319
                                    low
                                               dex
                                                                56561.0 0.58
## 92 MPIPSYKL_003785
                         ATAC
                                   high
                                                           1
                                                               217473.5 0.29
                                               veh
## 93 MPIPSYKL_001048
                                   high
                         ATAC
                                               dex
                                                               101095.5 0.60
## 94 MPIPSYKL_002095
                         ATAC
                                    low
                                                               809162.0 0.36
                                               dex
## 95 MPIPSYKL_002088
                         ATAC
                                    low
                                               veh
                                                               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
## 2 Treatment
                 dex
                           47
                                 veh
                                            48
                                                   3285
                                                              1809
```

Result:

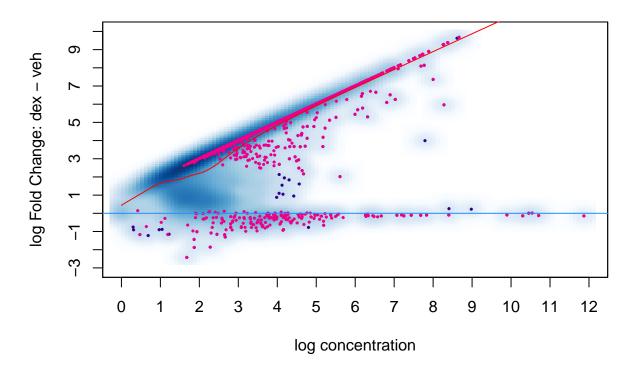
FDR = 5%:

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

EdgeR: 3285 of the 4781

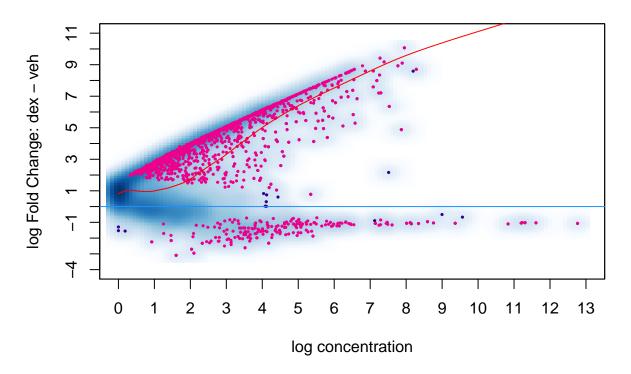
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2, bNormalized=F)

dex vs. veh (1809 FDR < 0.050)



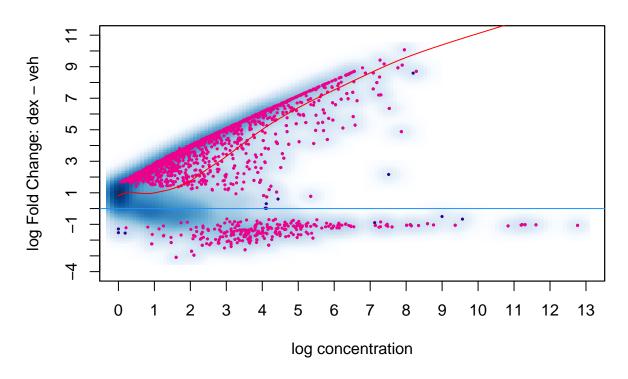
dba.plotMA(dba.dif.anal.norm, method = DBA_DESEQ2, contrast = 2)

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)</pre>
```

- ## Applying Blacklist/Greylists...
- ## Genome detected: Hsapiens.NCBI.GRCh38
- ## Applying blacklist...
- ## Removed: 3 of 4784 intervals.
- $\ensuremath{\mbox{\#\#}}$ No control reads specified, unable to generate greylist.
- ## Removed 3 (of 4784) consensus peaks.
- ## Normalize edgeR with defaults...
- ## Normalize DESeq2 with defaults...
- ## Analyzing...

##	95	Samples,	4781	sites in	matrix (4	784 total)	:	
##		-	I	D Factor	Condition	Treatment	Replicate	Reads FRiP
##	1	MPIPSYKL_	_00063	37 ATAC	low	veh	1	48646.5 0.48
##	2	MPIPSYKL_	00241	ATAC	low	veh	1	39876.0 0.41
##	3	MPIPSYKL_	_00378	37 ATAC	high	dex	2	122369.5 0.60
##	4	MPIPSYKL_	_00170	1 ATAC	high	veh	1	252860.5 0.28
##	5	MPIPSYKL_	_00323	31 ATAC	high	veh	1	32347.0 0.38
##	6	MPIPSYKL_	_00417	1 ATAC	low	dex	2	173266.5 0.61
##	7	MPIPSYKL_	_00260	4 ATAC	low	dex	2	73592.0 0.63
##	8	MPIPSYKL_	_00405	4 ATAC	low	dex	2	133546.5 0.65
##	9	MPIPSYKL_	_00126	S3 ATAC	high	veh	1	25906.5 0.46
##		MPIPSYKL_	-		high	veh	1	38769.0 0.35
##		MPIPSYKL_	-		high	veh	1	44678.0 0.41
##		MPIPSYKL_	-		high	dex	2	26133.0 0.45
##		MPIPSYKL_	_		low	veh	1	44139.0 0.42
##		MPIPSYKL_	_			dex	2	1093528.0 0.38
##		MPIPSYKL_	-			veh	1	136884.0 0.33
##		MPIPSYKL_	-		C	veh	1	34792.5 0.37
##		MPIPSYKL_	_		C	veh	1	97502.5 0.34
##		MPIPSYKL_	-			veh	1	59254.5 0.34
##		MPIPSYKL_	_			dex	2	80213.0 0.70
##		MPIPSYKL_	-			veh	1	29695.5 0.35
##		MPIPSYKL_	_		0	dex	2	137986.5 0.72
##		MPIPSYKL_	_		0	dex	2	120145.0 0.63
##		MPIPSYKL_			C	dex		1128909.5 0.51
##		MPIPSYKL_	_			veh	1	136983.5 0.34
##		MPIPSYKL_	_			veh	1	50702.5 0.50
##		MPIPSYKL_	_		0	veh	1	89583.0 0.32
##		MPIPSYKL_	_		C	veh	1	35730.5 0.42
##		MPIPSYKL_				veh	1	33399.5 0.39
##		MPIPSYKL_MPIPSYKL_	-		O	dex dex	2 2	44901.0 0.41 79331.0 0.60
##		MPIPSYKL	_			veh	1	32884.0 0.44
##		MPIPSYKL	_			dex	2	41535.5 0.51
##		MPIPSYKL_	_			veh	1	38420.0 0.42
##		MPIPSYKL_	_		0	dex	2	315901.5 0.67
		MPIPSYKL_	_			veh	1	26032.0 0.47
		MPIPSYKL				veh	1	174999.5 0.32
		MPIPSYKL	_			dex	2	80250.5 0.49
		MPIPSYKL			_	dex	2	88498.0 0.65
		MPIPSYKL_	_		_	dex	2	113871.0 0.45
		MPIPSYKL_	_		_	veh	1	52387.5 0.35
		MPIPSYKL_	_			veh	1	32213.5 0.39
		MPIPSYKL	_		•	dex	2	56650.5 0.53
		MPIPSYKL			high	dex	2	45694.0 0.56
		MPIPSYKL			high	dex	2	65412.0 0.55
##	45	MPIPSYKL	00393	34 ATAC	high	dex	2	357234.0 0.60
		MPIPSYKL			•	veh	1	38184.5 0.34
##	47	MPIPSYKL	00421	.5 ATAC	high	veh	1	31424.0 0.48
##	48	MPIPSYKL	00424	6 ATAC	_	dex	2	86453.5 0.62
##	49	MPIPSYKL_	_00111	.3 ATAC	high	veh	1	36781.5 0.35
##	50	MPIPSYKL_	_00154	7 ATAC	high	dex	2	20935.0 0.57

```
## 51 MPIPSYKL 001667
                          ATAC
                                    high
                                                                  33561.5 0.38
                                                veh
                                                              1
                                                             2
## 52 MPIPSYKL_000637
                          ATAC
                                     low
                                                                  84438.0 0.62
                                                dex
                                                                  52700.0 0.59
## 53 MPIPSYKL 001386
                          ATAC
                                    high
                                                dex
                                                              2
## 54 MPIPSYKL_002468
                          ATAC
                                                              2
                                                                  92503.5 0.71
                                    high
                                                dex
## 55 MPIPSYKL 002088
                          ATAC
                                     low
                                                dex
                                                              2
                                                                 178600.5 0.63
  56 MPIPSYKL 003013
                          ATAC
                                    high
                                                              1
                                                                 101447.5 0.35
                                                veh
                                                              2
## 57 MPIPSYKL 000972
                          ATAC
                                      low
                                                dex
                                                                 112234.5 0.43
## 58 MPIPSYKL 001048
                          ATAC
                                    high
                                                veh
                                                              1
                                                                  95489.5 0.27
## 59 MPIPSYKL_001389
                          ATAC
                                      low
                                                              1
                                                                  50559.5 0.44
                                                veh
## 60 MPIPSYKL_003785
                          ATAC
                                    high
                                                dex
                                                              2
                                                                 378592.5 0.43
## 61 MPIPSYKL_003155
                          ATAC
                                     low
                                                              1
                                                                  41054.5 0.36
                                                veh
  62 MPIPSYKL_001886
                          ATAC
                                     low
                                                veh
                                                              1
                                                                  98282.5 0.37
                                    high
   63 MPIPSYKL_003787
                          ATAC
                                                              1
                                                                  27634.0 0.45
                                                veh
   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
                                                              1
                                                                 199912.5 0.31
                                                veh
                                                              2
## 67 MPIPSYKL_001389
                          ATAC
                                      low
                                                                 157832.0 0.74
                                                dex
                                                              2
## 68 MPIPSYKL 003155
                          ATAC
                                      low
                                                                  77820.0 0.67
                                                dex
## 69 MPIPSYKL_000708
                          ATAC
                                                              2
                                      low
                                                                  96744.0 0.57
                                                dex
## 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
                                                              2
                                                                  62343.5 0.62
                          ATAC
                                    high
                                                dex
## 73 MPIPSYKL_002604
                          ATAC
                                      low
                                                              1
                                                                  25425.5 0.47
                                                veh
                                                              1
## 74 MPIPSYKL 002533
                          ATAC
                                    high
                                                veh
                                                                  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
                                     low
                                                                  66633.5 0.39
                          ATAC
                                                veh
                                                              1
                                                              2
  78 MPIPSYKL_003472
                          ATAC
                                    high
                                                                  37347.5 0.45
                                                dex
  79 MPIPSYKL_001386
                                                              1
                                                                  35902.5 0.42
                          ATAC
                                    high
                                                veh
## 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
                                                              2
                                                                  95701.5 0.43
                                                dex
                                                              2
## 83 MPIPSYKL_002383
                          ATAC
                                      low
                                                                  67016.0 0.46
                                                dex
## 84 MPIPSYKL_002658
                          ATAC
                                      low
                                                              1
                                                                  21971.5 0.48
                                                veh
  85 MPIPSYKL 001766
                          ATAC
                                                              2
                                                                 343078.0 0.33
                                      low
                                                dex
  86 MPIPSYKL_002852
                                                              2
                          ATAC
                                     low
                                                dex
                                                                 576255.5 0.64
## 87 MPIPSYKL 001263
                          ATAC
                                    high
                                                dex
                                                                 112898.5 0.69
## 88 MPIPSYKL_004198
                          ATAC
                                                              1
                                                                  19719.5 0.53
                                      low
                                                veh
## 89 MPIPSYKL_003353
                                                              2
                                                                 485290.5 0.52
                          ATAC
                                      low
                                                dex
                                                              2
## 90 MPIPSYKL_001667
                          ATAC
                                    high
                                                                 158705.5 0.47
                                                dex
## 91 MPIPSYKL 002319
                          ATAC
                                     low
                                                dex
                                                                  56561.0 0.58
## 92 MPIPSYKL_003785
                          ATAC
                                                              1
                                                                 217473.5 0.29
                                    high
                                                veh
## 93 MPIPSYKL 001048
                          ATAC
                                    high
                                                dex
                                                              2
                                                                 101095.5 0.60
  94 MPIPSYKL_002095
                                                              2
                                                                 809162.0 0.36
                          ATAC
                                      low
                                                dex
## 95 MPIPSYKL_002088
                          ATAC
                                      low
                                                                 106472.0 0.33
                                                 veh
##
## Design: [~Condition + Treatment] | 2 Contrasts:
        Factor Group Samples Group2 Samples2 DB.edgeR DB.DESeq2
## 1 Condition
                  low
                            47
                                 high
                                             48
                                                        4
                                                                   0
## 2 Treatment
                            47
                                             48
                                                     3270
                                                                1766
                  dex
                                  veh
```

Result:

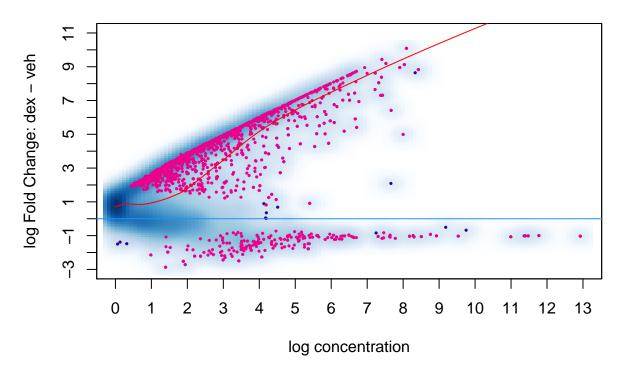
FDR = 5%:

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

EdgeR: 3270 of the 4781

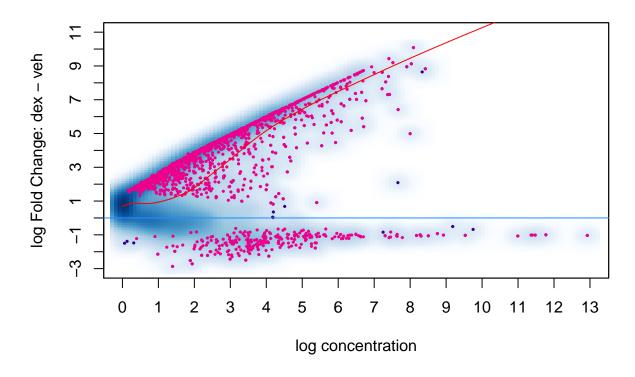
dba.plotMA(dba.dif.anal, method = DBA_DESEQ2, contrast = 2, bNormalized=T)

dex vs. veh (1766 FDR < 0.050)



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

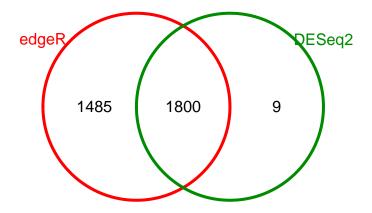
dex vs. veh (2035 FDR < 0.100)



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

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

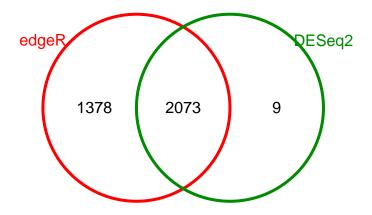
Binding Site Overlaps, FDR = 5%



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 =</pre>
```

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$

# 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$config$th <- 0.10</pre>
```

9. Retrieve the dif bound sites

```
## GRanges object with 6 ranges and 101 metadata columns:
         seqnames
##
                               ranges strand |
                                                   Conc Conc_dex Conc_veh
            <Rle>
##
                            <IRanges> <Rle> | <numeric> <numeric> <numeric>
                8 11545707-11546107
                                                              8.43
##
    4102
                                                   7.41
                                                                          0
    1431
               15
                    31365735-31366135
                                           * |
                                                    8.09
                                                              9.11
##
```

```
2274
                                                                    8.54
##
                      43131375-43131775
                                                         7.52
##
     3688
                  6
                      35727909-35728309
                                               * |
                                                         8.42
                                                                    9.44
     3577
                                                         6.93
                                                                    7.94
##
                  6
                      12369970-12370370
                                               * |
##
     2500
                                                         7.22
                                                                    8.24
                  2 201126779-201127179
                                               * |
##
                Fold
                       p-value
                                       FDR MPIPSYKL_003787 MPIPSYKL_004171
##
           <numeric> <numeric> <numeric>
                                                 <numeric>
                                                                   <numeric>
##
                9.43 5.67e-194 2.16e-190
                                                     360.28
                                                                      397.85
                                                     688.27
##
     1431
               10.09 4.17e-186 6.92e-183
                                                                      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>
##
                    548.03
                                      447.85
     4102
                                                       403.00
                                                                        284.77
##
     1431
                    899.09
                                      649.16
                                                       605.57
                                                                        419.98
##
     2274
                                     409.07
                                                                        311.07
                    624.28
                                                       395.58
##
     3688
                   1146.90
                                      801.52
                                                       712.68
                                                                        471.90
##
     3577
                    409.83
                                      363.82
                                                       338.31
                                                                        177.99
##
     2500
                                      433.08
                                                       373.31
                    465.43
                                                                        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
                                                       217.93
                                                                        266.76
                                      340.89
##
     2500
                    342.99
                                      350.63
                                                       303.18
                                                                        330.14
##
          MPIPSYKL 001547 MPIPSYKL 000637 MPIPSYKL 001386 MPIPSYKL 002468
##
                 <numeric>
                                  <numeric>
                                                    <numeric>
                                                                     <numeric>
```

0

0

0

##	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>	<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>	<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		
##		<numeric></numeric>	- <numeric></numeric>	- <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>	<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>	<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>	<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_001263		
##		<numeric></numeric>	<numeric></numeric>	<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 2500	0	0	0	0
##	2500	•	MPIPSYKL_003331	-	•
##		<pre><rpre></rpre></pre>	<pre></pre>	<pre><rpre><rpre></rpre></rpre></pre>	<pre><rp><pre><numeric></numeric></pre></rp></pre>
##	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>	<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_004251	_	_
##	4400	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>
##	4102	0	0	0	0
##	1431	0	0	0	0
## ##	2274 3688	0	0	0	0
##	3577	0	0	0	0
##	2500	0	0	0	0
##	2000	•	MPIPSYKL_003472	•	•
##		<pre><numeric></numeric></pre>	<pre><numeric></numeric></pre>	<pre><numeric></numeric></pre>	<pre><numeric></numeric></pre>
##	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>	<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 MDTDGVVI 001667	0 MDTDGVVI 002012	0 MDTDQVVI 001049	0 MDTDQVVI 001290
## ##			MPIPSYKL_003013		
##	4102	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>
##	1431	0	0	0	0
##	2274	0	0	0	0
##	3688	0	0	0	0
		ű	· ·	J	· ·

##	3577	0	0	0	0
##	2500	0	0	0	0
##		MPIPSYKL_003155	MPIPSYKL_001886	MPIPSYKL_003787	MPIPSYKL_004246
##		<numeric></numeric>	<numeric></numeric>	<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_002613		
##	4.4.0.0	<numeric></numeric>	<numeric></numeric>	<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 MDTDQVVI 000E33	0 MDTDQVVI 001E47	0 MDTDCVVI 004100	0 MDTDCVVI 001206
## ##			MPIPSYKL_001547	<pre>mpipsikL_004100 <numeric></numeric></pre>	<pre>mplpSikL_001386 <numeric></numeric></pre>
	4102	<numeric></numeric>	<numeric></numeric>	O Chamerica	O Chamerics
##	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
##	2000		MPIPSYKL_002658		
##		<numeric></numeric>	<numeric></numeric>	<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></numeric>			
##	4102	0			
##		0			
##	1431	O			
##	2274	0			
	2274 3688	0			
##	2274 3688 3577	0 0 0			
	2274 3688	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</pre>
    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",</pre>
                                                                                                       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, KI270311
##
##
                   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI270727.1
                  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 oth
                   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI270311
##
                   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI270727.1
##
                  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 oth
                   - in 'x': GL000008.2, GL000214.1, GL000224.1, KI270303.1, KI270304.1, KI270310.1, KI270311.1, KI270311
##
                   - in 'y': GL000205.2, GL000213.1, GL000218.1, GL000219.1, KI270721.1, KI270726.1, KI270727.1, KI270727.1
##
##
                  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 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 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).
```

dex.anno.report

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

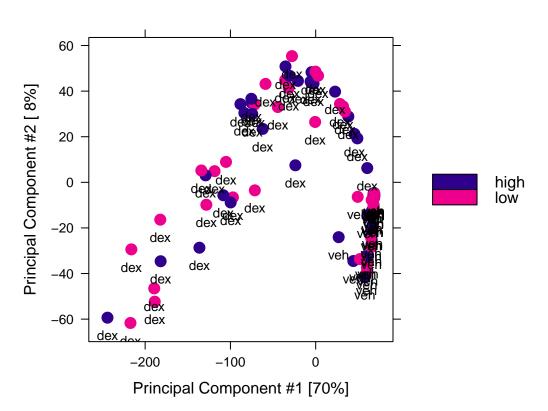
12. Extract annoted count matrix for RASQUAL

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

13. Plotting

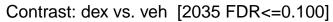
13.1 PCA

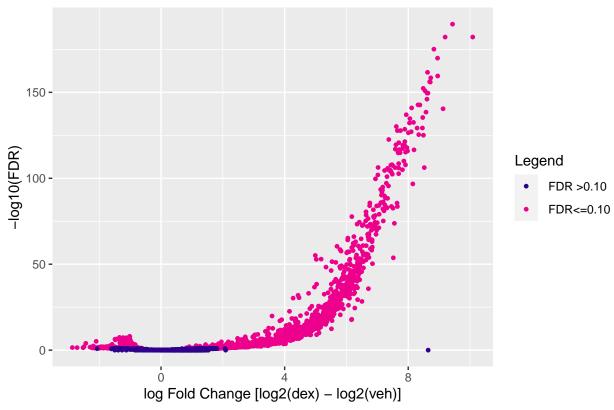
PCA: Condition



13.2. Volcano

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

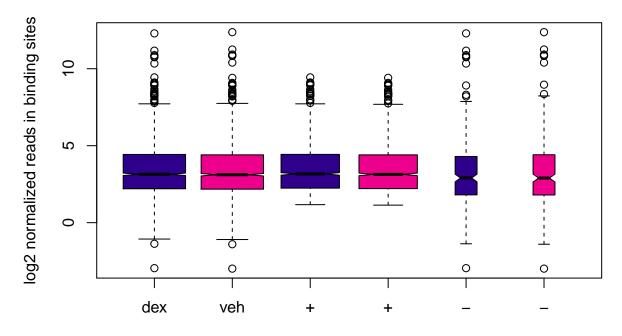




13.3. Boxplot

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

dex vs. veh



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

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_echriched.c

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_rasq

# 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.
```