Custom array sequence generation

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Wed Jan 13 10:18:35 2016

This script generates all AA unique AA sequences for the CustomArray production

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(parallel))
suppressPackageStartupMessages(library(GeneGA))
suppressPackageStartupMessages(library(devtools))
suppressPackageStartupMessages(library(Hmisc))
```

Loading source files

```
source(file.path("functions", "AAtoDNA.R"))

allSequences <- readFasta("input/DNA-lib_RetrogradeTransport.fasta")
AAlist <- data.frame(Class=character(),
                     Family=character(),
                     Strain=character(),
                     Note=character(),
                     Number=character(),
                     Name=character(),
                     AAfragment=character(),
                     stringsAsFactors = FALSE)

#allSequences <- allSequences[124:129]

strt<-Sys.time()
for (i in 1:length(allSequences)){
  thisID <- as.character(ShortRead::id(allSequences[i]))
  thisSeq <- sread(allSequences[i])
  thisAA <- Biostrings::translate(thisSeq, genetic.code=GENETIC_CODE, if.fuzzy.codon="solve")
  AAlist[i,c("Class","Family","Strain","Note",
             "Number","Name","AAfragment")] <- c(BBmisc::explode(thisID, sep=","),as.character(thisAA))
}
```

The generateFragments function

```
generateFragments <- function(minLength,maxLength,frequency=1) {
#Generate a table to store all AA sequences
fragList <- data.frame(Class=character(),
                       Family=character(),
                       Strain=character(),
                       Note=character(),
                       Number=character(),
                       Name=character(),
                       AAstart=integer(),
```

```
                        AAstop=integer(),
                        AAfragment=character(),
                        stringsAsFactors = FALSE)


makeAllFrags <- function(k){
    thisFullAA <- AAlist[k,"AAfragment"]
    count = length(fragList[,1])+1
    for (l in seq(1,(width(thisFullAA)-minLength),frequency)){
      for (m in (l+minLength-1):(min(l+maxLength-1,width(thisFullAA)))){
      #Truncate string to the relevant fragment:
      thisFragment <- substr(thisFullAA,l,m)
      #Take away any sequence that starts with a start codon ATG:
      if (substr(thisFragment,1,1)!="M") {
      fragList[count,c("Class","Family","Strain","Note","Number",
                       "Name","AAstart","AAstop",
                       "AAfragment")] <- c(AAlist[k,c("Class","Family","Strain",
                                                      "Note","Number","Name")],l,m,thisFragment)
      ## Inserts the fragment with information into the new data frame
      count <- count +1
      }
      }
    }
    return(fragList)
  }

fragList <- do.call(rbind,mclapply(1:length(AAlist[,1]),makeAllFrags,
                                   mc.preschedule = TRUE, mc.cores = detectCores()))

#Control if any sequences contain non-AA characters and save them into a separate list
discardList <- fragList[grep("[[:punct:]|X]",fragList[,"AAfragment"]),]

#Remove any sequence containing non AA characters
fragList <- fragList[grep("[[:punct:]|X]",fragList[,"AAfragment"], invert = TRUE),]


#Sort the fragments, find unique strings and count number of duplicates
sortedFragments <- rev(sort(table(fragList[,"AAfragment"])))

#Run the AAtoDNA function to convert all AA sequences to human codon-optimized DNA sequences
row.names(sortedFragments) <- mclapply(row.names(sortedFragments), fullOPT=FALSE, species="hsa",
                        AAtoDNA, mc.preschedule = TRUE, mc.set.seed = TRUE,
                        mc.silent = FALSE, mc.cores = detectCores(), mc.cleanup = TRUE) #

sortedFragments <- sortedFragments[order(row.names(sortedFragments))]

return(sortedFragments)
}
```

Execution of the function

```
sortedFragments.14aa <- generateFragments(14,14,1)
```

Add the overhangs for amplication PCR and Gibson assembly into the AAV plasmid

```
fivePrime <- tolower("AACCTCCAGAGAGGCAACGCT")
threePrime <- tolower("GCCAGACAAGCAGCTACCGCA")
row.names(sortedFragments.14aa) <- paste(fivePrime,row.names(sortedFragments.14aa),
                                         threePrime, sep = "")

sortedFragments.14aa.G4S <- generateFragments(14,14,3)
sortedFragments.14aa.A5 <- sortedFragments.14aa.G4S
```

Add the overhangs including G4S spacers for amplication PCR and Gibson assembly into the AAV plasmid

```
fivePrime <- tolower("AACCTCCAGAGAGGCAACGGAGGCGGAGGAAGT")
threePrime <- tolower("GGAGGCGGCGGAAGCAGACAAGCAGCTACCGCA")
row.names(sortedFragments.14aa.G4S) <- paste(fivePrime,row.names(sortedFragments.14aa.G4S),
                                             threePrime, sep = "")
```

Add the overhangs including A5 spacers for amplication PCR and Gibson assembly into the AAV plasmid

```
fivePrime <- tolower("AACCTCCAGAGAGGCAACGCTGCTGCAGCAGCC")
threePrime <- tolower("GCAGCTGCAGCTGCCAGACAAGCAGCTACCGCA")
row.names(sortedFragments.14aa.A5) <- paste(fivePrime,row.names(sortedFragments.14aa.A5),
                                            threePrime, sep = "")
```

Generate 22aa fragments

```
sortedFragments.22aa <- generateFragments(22,22,3)
```

Add the overhangs for amplication PCR and Gibson assembly into the AAV plasmid

```
fivePrime <- tolower("AACCTCCAGAGAGGCAACGCT")
threePrime <- tolower("GCCAGACAAGCAGCTACCGCA")
row.names(sortedFragments.22aa) <- paste(fivePrime,row.names(sortedFragments.22aa),
                                         threePrime, sep = "")
```

Merge all separate fragment lists into one complete list

```
sortedFragments <- c(sortedFragments.22aa,sortedFragments.14aa,
                     sortedFragments.14aa.A5,sortedFragments.14aa.G4S)

print(paste("Number of unique fragments:",length(unique(names(sortedFragments))), sep=" "))
```

```
## [1] "Number of unique fragments: 92358"
```

```
write.table(c("Sequence",unique(names(sortedFragments))),
            "data/SortedFragments_all.txt",row.names=F,col.names=F,quote=F,sep="\t")

print(Sys.time()-strt)
```

```
## Time difference of 2.857151 mins
```

```
devtools::session_info()
```

```
## Session info -------------------------------------------------------------
```

```
##  setting  value
##  version  R version 3.2.3 (2015-12-10)
##  system   x86_64, linux-gnu
##  ui       X11
##  language en_US:en
##  collate  en_US.UTF-8
##  tz       <NA>
##  date     2016-01-13
```

```
## Packages -------------------------------------------------------------------
```

```
##  package              * version date       source
##  acepack                1.3-3.3 2014-11-24 CRAN (R 3.2.0)
##  ade4                 * 1.7-3   2015-11-22 CRAN (R 3.2.2)
##  BBmisc                 1.9     2015-02-03 CRAN (R 3.2.0)
##  Biobase              * 2.30.0  2015-11-26 Bioconductor
##  BiocGenerics         * 0.16.1  2015-11-26 Bioconductor
##  BiocParallel         * 1.4.0   2015-11-26 Bioconductor
##  Biostrings           * 2.38.2  2015-11-26 Bioconductor
##  bitops                 1.0-6   2013-08-17 CRAN (R 3.2.0)
##  checkmate              1.6.3   2015-10-23 CRAN (R 3.2.2)
##  cluster                2.0.3   2015-07-21 CRAN (R 3.2.2)
##  colorspace             1.2-6   2015-03-11 CRAN (R 3.2.0)
##  devtools             * 1.9.1   2015-09-11 CRAN (R 3.2.2)
##  digest                 0.6.8   2014-12-31 CRAN (R 3.2.0)
##  evaluate               0.8     2015-09-18 CRAN (R 3.2.2)
##  foreign                0.8-66  2015-08-19 CRAN (R 3.2.2)
##  Formula              * 1.2-1   2015-04-07 CRAN (R 3.2.0)
##  futile.logger          1.4.1   2015-04-20 CRAN (R 3.2.0)
##  futile.options         1.0.0   2010-04-06 CRAN (R 3.2.0)
##  GeneGA               * 1.20.0  2015-11-26 Bioconductor
##  GenomeInfoDb         * 1.6.1   2015-11-26 Bioconductor
##  GenomicAlignments    * 1.6.1   2015-11-26 Bioconductor
##  GenomicRanges        * 1.22.1  2015-11-26 Bioconductor
##  ggplot2              * 1.0.1   2015-03-17 CRAN (R 3.2.0)
##  gridExtra              2.0.0   2015-07-14 CRAN (R 3.2.2)
##  gtable                 0.1.2   2012-12-05 CRAN (R 3.2.0)
##  hash                 * 2.2.6   2013-02-21 CRAN (R 3.2.0)
##  Hmisc                * 3.17-0  2015-09-21 CRAN (R 3.2.2)
##  htmltools              0.2.6   2014-09-08 CRAN (R 3.2.0)
##  hwriter                1.3.2   2014-09-10 CRAN (R 3.2.0)
##  IRanges              * 2.4.4   2015-11-26 Bioconductor
##  knitr                * 1.11    2015-08-14 CRAN (R 3.2.2)
##  lambda.r               1.1.7   2015-03-20 CRAN (R 3.2.0)
##  lattice              * 0.20-33 2015-07-14 CRAN (R 3.2.2)
##  latticeExtra           0.6-26  2013-08-15 CRAN (R 3.2.0)
##  magrittr               1.5     2014-11-22 CRAN (R 3.2.2)
##  MASS                   7.3-45  2015-11-10 CRAN (R 3.2.2)
##  memoise                0.2.1   2014-04-22 CRAN (R 3.2.2)
##  munsell                0.4.2   2013-07-11 CRAN (R 3.2.0)
##  nnet                   7.3-11  2015-08-30 CRAN (R 3.2.2)
##  plyr                   1.8.3   2015-06-12 CRAN (R 3.2.2)
##  proto                  0.3-10  2012-12-22 CRAN (R 3.2.0)
##  RColorBrewer           1.1-2   2014-12-07 CRAN (R 3.2.0)
##  Rcpp                   0.12.2  2015-11-15 CRAN (R 3.2.2)
##  reshape2               1.4.1   2014-12-06 CRAN (R 3.2.0)
##  rmarkdown              0.8.1   2015-10-10 CRAN (R 3.2.2)
```

```
##  rpart                  4.1-10  2015-06-29 CRAN (R 3.2.2)
##  Rsamtools            * 1.22.0  2015-11-26 Bioconductor
##  S4Vectors            * 0.8.3   2015-11-26 Bioconductor
##  scales                 0.3.0   2015-08-25 CRAN (R 3.2.2)
##  seqinr               * 3.1-3   2014-12-17 CRAN (R 3.2.0)
##  ShortRead            * 1.28.0  2015-11-26 Bioconductor
##  stringi                1.0-1   2015-10-22 CRAN (R 3.2.2)
##  stringr                1.0.0   2015-04-30 CRAN (R 3.2.2)
##  SummarizedExperiment * 1.0.1   2015-11-26 Bioconductor
##  survival             * 2.38-3  2015-07-02 CRAN (R 3.2.2)
##  XVector              * 0.10.0  2015-11-26 Bioconductor
##  yaml                   2.1.13  2014-06-12 CRAN (R 3.2.0)
##  zlibbioc               1.16.0  2015-11-26 Bioconductor
```

Library analysis output

Tomas Bjorklund

Wed Jan 13 10:21:35 2016

This workflow brings together FastQ files containing barcodes and the gene fragments synthesized with the CustomArray. Requires bbmap2. The fragments are then suitable for alignment to reference sequences using Bowtie2.

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(parallel))
suppressPackageStartupMessages(library(doParallel))
suppressPackageStartupMessages(library(data.table))
suppressPackageStartupMessages(library(devtools))
suppressPackageStartupMessages(library(Hmisc))

opts_chunk$set(fig.width = 7.5, fig.height = 8)
opts_chunk$set(comment = NA)

config <- read.table("input/config.txt", header = FALSE, skip = 0, sep="\t",
                     stringsAsFactors = FALSE, fill=TRUE)
colnames(config) <- c("Parameter", "Value")
```

Sequencing files

```
dataDir <- config$Value[1]
in.name.P5 <- file.path(dataDir, config$Value[2])
in.name.P7 <- file.path(dataDir, config$Value[3])
name.out <- config$Value[4]
paired.alignment <- as.logical(config$Value[5])
```

Analysis parameters

```
knitr::kable(config, format = "markdown")
```

Parameter	Value
dataDir	seqFiles/DNA
in.name.P5	psc-lib-1-2UndetOldSort_S1_L001_R1_001.fastq.gz
in.name.P7	psc-lib-1-2UndetOldSort_S1_L001_R2_001.fastq.gz
name.out	AAVlibrary_complete
paired.alignment	TRUE
run.subset	FALSE
max.cores	32
subset.count	250000

```
run.subset <- as.logical(config$Value[6])
max.cores <- as.integer(config$Value[7])
subset.count <- as.integer(config$Value[8])

strt<-Sys.time()
```

Selection of real amplicons

```
# This section searches the sequencing file and only select the files with valid amplicons
out.name.P5 <- tempfile(pattern = "P5_", tmpdir = tempdir(), fileext = ".fastq.gz")
out.name.P7 <- tempfile(pattern = "P7_", tmpdir = tempdir(), fileext = ".fastq.gz")
command.args <- paste("-Xmx12g overwrite=true k=15 rcomp=f skipr2=t qhdist=0 maskmiddle=f",
                      " hammingdistance=2 findbestmatch=f ordered=t threads=",detectCores(),
                      " in=", in.name.P5,
                      " in2=", in.name.P7,
                      " outm=", out.name.P5,
                      " outm2=", out.name.P7,
                      " fliteral=", "GTATGTTGTTCTGGAGCGGGAGGGTGCTATTTTGCCTAGCGATAA", sep = "")

sys.out <- system2(path.expand("~/bbmap/bbduk2.sh"), args=command.args, stdout=TRUE, stderr=TRUE)

sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("bbduk2 Identification of real amplicons")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[3:lengthOut,], format = "markdown")
```

	bbduk2 Identification of real amplicons
3	
4	BBDuk2 version 34.79
5	Set ORDERED to true
6	Set threads to 32
7	k=15
8	hamming distance=2
9	kfiltering using 1 literal.
10	
11	Initial:
12	Memory: free=12090m, used=258m
13	
14	Added 30721 kmers; time: 0.083 seconds.
15	Memory: free=11639m, used=709m
16	
17	Input is being processed as paired
18	Started output streams: 0.103 seconds.
19	Processing time: 84.814 seconds.

bbduk2 Identification of real amplicons

```
20
21 Input: 23191088 reads 3490215687 bases.
22 Contaminants: 23095890 reads (99.59%) 3475908371 bases (99.59%)
23 Result: 95198 reads (0.41%) 14307316 bases (0.41%)
24
25 Time: 85.009 seconds.
26 Reads Processed: 23191k 272.81k reads/sec
27 Bases Processed: 3490m 41.06m bases/sec
```

```
in.name.P5 <- out.name.P5
in.name.P7 <- out.name.P7
```

Extraction of a subset

```
if (run.subset){
  suppressWarnings(sampler <- FastqSampler(gsub("([\\])", "", in.name.P5), subset.count,
                                           readerBlockSize=1e9, ordered = TRUE))
  set.seed(123); tmp.P5 <- yield(sampler)
  in.name.P5 <- tempfile(pattern = "P5_", tmpdir = tempdir(), fileext = ".fastq.gz")
  writeFastq(tmp.P5,in.name.P5, compress=TRUE)
  rm(tmp.P5)
  suppressWarnings(sampler <- FastqSampler(gsub("([\\])", "", in.name.P7), subset.count,
                                           readerBlockSize=1e9, ordered = TRUE))
  set.seed(123); tmp.P7 <- yield(sampler)
  in.name.P7 <- tempfile(pattern = "P7_", tmpdir = tempdir(), fileext = ".fastq.gz")
  writeFastq(tmp.P7,in.name.P7, compress=TRUE)
  rm(tmp.P7)
}

output.Reads <- as.integer(system(paste("gunzip -c ",shQuote(gsub("([\\])", "", in.name.P5)),
                                        " | echo $((`wc -l`/4)) 2>&1", sep = ""), intern = TRUE,
                                  ignore.stdout = FALSE)) #Stores the read count utilized
print(paste("Utilized sequences:", output.Reads))
```

```
[1] "Utilized sequences: 11547945"
```

Extraction of barcodes

```
out.name.P5 <- tempfile(pattern = "BC_", tmpdir = tempdir(), fileext = ".fastq.gz")

sys.out <- system(paste("~/bbmap/bbduk2.sh overwrite=true k=18 mink=18 hammingdistance=2 findbestmatch=t ",
                        "rcomp=f findbestmatch=f qhdist=1 minavgquality=0 maxns=0 minlength=18 ",
                        "maxlength=22 threads=", detectCores()," in=", shQuote(in.name.P5),
                        " out=", out.name.P5," lliteral=", "GGCCTAGCGGCCGCTTTACTT",
                        " rliteral=", "ATAACTTCGTATAATGTATGC",
```

```
                        " 2>&1", sep = ""), intern = TRUE, ignore.stdout = FALSE)
sys.out <- as.data.frame(sys.out)

in.name.P5 <- out.name.P5


colnames(sys.out) <- c("bbduk2 Extraction of barcodes")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[3:lengthOut,], format = "markdown")
```

	bbduk2 Extraction of barcodes
3	
4	BBDuk2 version 34.79
5	Set threads to 32
6	k=18
7	maskMiddle=true
8	hamming distance=2
9	right-ktrimming using 1 literal.
10	left-ktrimming using 1 literal.
11	
12	Initial:
13	Memory: free=77148m, used=2496m
14	
15	Added 5104 kmers; time: 0.047 seconds.
16	Memory: free=74239m, used=5405m
17	
18	Added 5104 kmers; time: 0.001 seconds.
19	Memory: free=74239m, used=5405m
20	
21	Input is being processed as unpaired
22	Started output streams: 0.018 seconds.
23	Processing time: 381.797 seconds.
24	
25	Input: 11547945 reads 1738273591 bases.
26	KTrimmed: 23030031 reads (199.43%) 1505373909 bases (86.60%)
27	Low quality discards: 6 reads (0.00%) 122 bases (0.00%)
28	Result: 11374106 reads (98.49%) 227257668 bases (13.07%)
29	
30	Time: 381.875 seconds.
31	Reads Processed: 11547k 30.24k reads/sec
32	Bases Processed: 1738m 4.55m bases/sec

```
rm(sys.out)

reads.BC <- readFastq(in.name.P5)
sread(reads.BC)
```

```
  A DNAStringSet instance of length 11374106
           width seq
       [1]    21 CTGTGCTGAATTACTTATACA
       [2]    20 GTAGGTATCTAGACTCCTTT
       [3]    20 GTACGTTGACTGGAAGCTGG
       [4]    20 GTGTGTAGCAGGATACGCGG
       [5]    20 CATGAGTTATTTGATGATAT
       ...   ... ...
[11374102]    20 GTATCAACGAATGCGTCATT
[11374103]    20 GTTTATGGGAGTGCTGCCGT
[11374104]    20 GTGGGATTGAAGGATGGTGT
[11374105]    20 GAGGGCTTATGTCAATGCGT
[11374106]    20 GATGGAGTGTAGGTGTGTTG
```

```
(unique.BCs <- unique(sread(reads.BC)))
```

```
  A DNAStringSet instance of length 3934570
          width seq
      [1]    21 CTGTGCTGAATTACTTATACA
      [2]    20 GTAGGTATCTAGACTCCTTT
      [3]    20 GTACGTTGACTGGAAGCTGG
      [4]    20 GTGTGTAGCAGGATACGCGG
      [5]    20 CATGAGTTATTTGATGATAT
      ...   ... ...
[3934566]    20 GTGTGTTCATTTCATTGTTT
[3934567]    20 GAACATGGCTTGGAGTGCGG
[3934568]    20 GTATCAACGAATGCGTCATT
[3934569]    20 GTTTATGGGAGTGCTGCCGT
[3934570]    20 GTGGGATTGAAGGATGGTGT
```

```
output.BCs <- length(unique.BCs)
print(paste("Utilized barcodes:", output.BCs))
```

```
[1] "Utilized barcodes: 3934570"
```

```
barcodeTable <- data.table(ID=as.character(ShortRead::id(reads.BC)), BC=as.character(sread(reads.BC)))
```

Extraction of fragments

```
out.name.P7 <- tempfile(pattern = "P7_", tmpdir = tempdir(), fileext = ".fastq.gz")
command.args <- paste("-Xmx12g overwrite=true k=18 mink=18 rcomp=f qhdist=1 maskmiddle=t",
                      " hammingdistance=2 findbestmatch=t minlength=38 maxlength=78 ordered=t ",
                      "threads=", detectCores(), " in=", in.name.P7, " out=", out.name.P7,
                      " lliteral=", "AGCAACCTCCAGAGAGGCAACG",
                      " rliteral=", "CAGACAAGCAGCTACCGCAGAT", sep = "")

sys.out <- system2(path.expand("~/bbmap/bbduk2.sh"), args=command.args, stdout=TRUE, stderr=TRUE) #
```

```
sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("bbduk2 extraction of fragments")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[3:lengthOut,], format = "markdown")
```

	bbduk2 extraction of fragments
3	
4	BBDuk2 version 34.79
5	Set ORDERED to true
6	Set threads to 32
7	k=18
8	maskMiddle=true
9	hamming distance=2
10	right-ktrimming using 1 literal.
11	left-ktrimming using 1 literal.
12	
13	Initial:
14	Memory: free=11959m, used=389m
15	
16	Added 6380 kmers; time: 0.252 seconds.
17	Memory: free=11508m, used=840m
18	
19	Added 6380 kmers; time: 0.003 seconds.
20	Memory: free=11508m, used=840m
21	
22	Input is being processed as unpaired
23	Started output streams: 0.633 seconds.
24	Processing time: 428.708 seconds.
25	
26	Input: 11547945 reads 1737634780 bases.
27	KTrimmed: 22321617 reads (193.30%) 1078844314 bases (62.09%)
28	Result: 10849152 reads (93.95%) 591375852 bases (34.03%)
29	
30	Time: 429.633 seconds.
31	Reads Processed: 11547k 26.88k reads/sec
32	Bases Processed: 1737m 4.04m bases/sec

```
in.name.P7 <- out.name.P7

out.name.P5 <- tempfile(pattern = "P5_", tmpdir = tempdir(), fileext = ".fastq.gz")
```

```
out.name.P7 <- tempfile(pattern = "P7_", tmpdir = tempdir(), fileext = ".fastq.gz")
out.name.P5_singlet <- tempfile(pattern = "P5_singlet_", tmpdir = tempdir(), fileext = ".fastq.gz")
out.name.P7_singlet <- tempfile(pattern = "P7_singlet_", tmpdir = tempdir(), fileext = ".fastq.gz")

sys.out <- system(paste("pairfq makepairs -c 'gzip' -f ", in.name.P5," -r ", in.name.P7,
                        " -fp ", out.name.P5, " -rp ", out.name.P7, " -fs ",
                        out.name.P5_singlet, " -rs ", out.name.P7_singlet,
                        " --stats 2>&1", sep = ""), intern = TRUE, ignore.stdout = FALSE)
sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("pairfq pair matching")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[1:lengthOut,], format = "markdown")
```

pairfq pair matching
========= pairfq version : 0.15 (completion time: Wed Jan 13 10:43:39 CET 2016)
Total forward reads (/tmp/RtmpnpI6ee/BC_195c76d2af98.fastq.gz) : 11374106
Total reverse reads (/tmp/RtmpnpI6ee/P7_195ccf10460.fastq.gz) : 10849152
Total forward paired reads (/tmp/RtmpnpI6ee/P5_195c3797e3fe.fastq.gz) : 10698072
Total reverse paired reads (/tmp/RtmpnpI6ee/P7_195c75634eb1.fastq.gz) : 10698072
Total forward unpaired reads (/tmp/RtmpnpI6ee/P5_singlet_195c585764.fastq.gz) : 676034
Total reverse unpaired reads (/tmp/RtmpnpI6ee/P7_singlet_195c34d6b7a3.fastq.gz) : 151080
Total paired reads : 21396144
Total unpaired reads : 827114

```
rm(sys.out)


system(paste("mv ", out.name.P5, " ./data/barcodes_", name.out, ".fastq.gz", sep=""))
system(paste("mv ", out.name.P7, " ./data/fragments_", name.out, ".fastq.gz", sep=""))

unlink(paste(tempdir(), "/*", sep = ""), recursive = FALSE, force = FALSE) #Cleanup of temp files

print("Total execution time:")
```

```
[1] "Total execution time:"
```

```
print(Sys.time()-strt)
```

```
Time difference of 22.0035 mins
```

```
devtools::session_info()
```

```
Session info -------------------------------------------------------------
```

```
 setting  value
```

```
 version  R version 3.2.3 (2015-12-10)
 system   x86_64, linux-gnu
 ui       X11
 language en_US:en
 collate  en_US.UTF-8
 tz       <NA>
 date     2016-01-13

Packages ------------------------------------------------------------------

 package              * version date       source
 acepack                1.3-3.3 2014-11-24 CRAN (R 3.2.0)
 Biobase              * 2.30.0  2015-11-26 Bioconductor
 BiocGenerics         * 0.16.1  2015-11-26 Bioconductor
 BiocParallel         * 1.4.0   2015-11-26 Bioconductor
 Biostrings           * 2.38.2  2015-11-26 Bioconductor
 bitops                 1.0-6   2013-08-17 CRAN (R 3.2.0)
 chron                  2.3-47  2015-06-24 CRAN (R 3.2.2)
 cluster                2.0.3   2015-07-21 CRAN (R 3.2.2)
 codetools              0.2-14  2015-07-15 CRAN (R 3.2.2)
 colorspace             1.2-6   2015-03-11 CRAN (R 3.2.0)
 data.table           * 1.9.6   2015-09-19 CRAN (R 3.2.2)
 devtools             * 1.9.1   2015-09-11 CRAN (R 3.2.2)
 digest                 0.6.8   2014-12-31 CRAN (R 3.2.0)
 doParallel           * 1.0.10  2015-10-14 CRAN (R 3.2.2)
 evaluate               0.8     2015-09-18 CRAN (R 3.2.2)
 foreach              * 1.4.3   2015-10-13 CRAN (R 3.2.2)
 foreign                0.8-66  2015-08-19 CRAN (R 3.2.2)
 formatR                1.2.1   2015-09-18 CRAN (R 3.2.2)
 Formula              * 1.2-1   2015-04-07 CRAN (R 3.2.0)
 futile.logger          1.4.1   2015-04-20 CRAN (R 3.2.0)
 futile.options         1.0.0   2010-04-06 CRAN (R 3.2.0)
 GenomeInfoDb         * 1.6.1   2015-11-26 Bioconductor
 GenomicAlignments    * 1.6.1   2015-11-26 Bioconductor
 GenomicRanges        * 1.22.1  2015-11-26 Bioconductor
 ggplot2              * 1.0.1   2015-03-17 CRAN (R 3.2.0)
 gridExtra              2.0.0   2015-07-14 CRAN (R 3.2.2)
 gtable                 0.1.2   2012-12-05 CRAN (R 3.2.0)
 highr                  0.5.1   2015-09-18 CRAN (R 3.2.2)
 Hmisc                * 3.17-0  2015-09-21 CRAN (R 3.2.2)
 htmltools              0.2.6   2014-09-08 CRAN (R 3.2.0)
 hwriter                1.3.2   2014-09-10 CRAN (R 3.2.0)
 IRanges              * 2.4.4   2015-11-26 Bioconductor
 iterators            * 1.0.8   2015-10-13 CRAN (R 3.2.2)
 knitr                * 1.11    2015-08-14 CRAN (R 3.2.2)
 lambda.r               1.1.7   2015-03-20 CRAN (R 3.2.0)
 lattice              * 0.20-33 2015-07-14 CRAN (R 3.2.2)
 latticeExtra           0.6-26  2013-08-15 CRAN (R 3.2.0)
 magrittr               1.5     2014-11-22 CRAN (R 3.2.2)
 MASS                   7.3-45  2015-11-10 CRAN (R 3.2.2)
 memoise                0.2.1   2014-04-22 CRAN (R 3.2.2)
 munsell                0.4.2   2013-07-11 CRAN (R 3.2.0)
 nnet                   7.3-11  2015-08-30 CRAN (R 3.2.2)
 plyr                   1.8.3   2015-06-12 CRAN (R 3.2.2)
 proto                  0.3-10  2012-12-22 CRAN (R 3.2.0)
 RColorBrewer           1.1-2   2014-12-07 CRAN (R 3.2.0)
 Rcpp                   0.12.2  2015-11-15 CRAN (R 3.2.2)
```

```
reshape2               1.4.1   2014-12-06 CRAN (R 3.2.0)
rmarkdown              0.8.1   2015-10-10 CRAN (R 3.2.2)
rpart                  4.1-10  2015-06-29 CRAN (R 3.2.2)
Rsamtools            * 1.22.0  2015-11-26 Bioconductor
S4Vectors            * 0.8.3   2015-11-26 Bioconductor
scales                 0.3.0   2015-08-25 CRAN (R 3.2.2)
ShortRead            * 1.28.0  2015-11-26 Bioconductor
stringi                1.0-1   2015-10-22 CRAN (R 3.2.2)
stringr                1.0.0   2015-04-30 CRAN (R 3.2.2)
SummarizedExperiment * 1.0.1   2015-11-26 Bioconductor
survival             * 2.38-3  2015-07-02 CRAN (R 3.2.2)
XVector              * 0.10.0  2015-11-26 Bioconductor
yaml                   2.1.13  2014-06-12 CRAN (R 3.2.0)
zlibbioc               1.16.0  2015-11-26 Bioconductor
```

Library identification

Tomas Bjorklund

Wed Jan 13 10:43:48 2016

This workflow aligns the library fragments to the full reference sequences using Bowtie2.

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(data.table))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(Hmisc))
suppressPackageStartupMessages(library(GeneGA))
suppressPackageStartupMessages(library(devtools))


opts_chunk$set(fig.width = 7.5, fig.height = 8)
opts_chunk$set(comment = NA)
```

Load sequences

```
LUT.dna <- read.table("data/SortedFragments_all.txt",
                      header = TRUE, skip = 0, sep="\t",stringsAsFactors = FALSE, fill=TRUE)
LUT.dna <- data.table(LUT.dna)
```

Remove constitutive backbone sequences

```
invisible(LUT.dna[,Sequence:=gsub("aacctccagagaggcaacg","",Sequence)])
invisible(LUT.dna[,Sequence:=gsub("cagacaagcagctaccgca","",Sequence)])
invisible(LUT.dna[,Sequence:=toupper(Sequence)])
setkey(LUT.dna, "Sequence")
LUT.dna <- unique(LUT.dna)
LUT.dna$Names <- LUT.dna$Sequence
```

Split sequences based on linker and length

```
LUT.14aaG4S <- LUT.dna[substr(LUT.dna$Sequence,1,14) == "GAGGCGGAGGAAGT"]
LUT.remaining <- LUT.dna[!(substr(LUT.dna$Sequence,1,14) == "GAGGCGGAGGAAGT")]
LUT.14aaA5 <- LUT.remaining[substr(LUT.remaining$Sequence,1,14) == "CTGCTGCAGCAGCC"]
LUT.remaining <- LUT.remaining[!(substr(LUT.remaining$Sequence,1,14) == "CTGCTGCAGCAGCC")]
LUT.22aa <- LUT.remaining[nchar(LUT.remaining$Sequence) == 70L &
                            substr(LUT.remaining$Sequence,1,2) == "CT"]
LUT.remaining <- LUT.remaining[!(nchar(LUT.remaining$Sequence) == 70L &
                                   substr(LUT.remaining$Sequence,1,2) == "CT")]
LUT.14aa <- LUT.remaining[nchar(LUT.remaining$Sequence) == 46L &
                            substr(LUT.remaining$Sequence,1,2) == "CT"]
rm(LUT.remaining)
LUT.dna[LUT.dna$Sequence %in% LUT.14aaG4S$Sequence,"Structure"] <- "14aaG4S"
```

```
LUT.dna[LUT.dna$Sequence %in% LUT.14aaA5$Sequence,"Structure"] <- "14aaA5"
LUT.dna[LUT.dna$Sequence %in% LUT.22aa$Sequence,"Structure"] <- "22aa"
LUT.dna[LUT.dna$Sequence %in% LUT.14aa$Sequence,"Structure"] <- "14aa"

save(LUT.dna,file = "data/LUTdna.rda")
```

Trim sequences

```
LUT.14aa$Sequence <- substr(LUT.14aa$Sequence,3,44)
LUT.14aaG4S$Sequence <- substr(LUT.14aaG4S$Sequence,15,56)
LUT.14aaA5$Sequence <- substr(LUT.14aaA5$Sequence,15,56)
LUT.22aa$Sequence <- substr(LUT.22aa$Sequence,3,68)
```

Save fasta files for Bowtie alignments

```
LUT.14aa.fa <- tempfile(pattern = "LUT_14aa_", tmpdir = tempdir(), fileext = "fa")
LUT.14aa.seq = ShortRead(DNAStringSet(LUT.14aa$Sequence), BStringSet(LUT.14aa$Names))
writeFasta(LUT.14aa.seq,LUT.14aa.fa)

LUT.14aaG4S.fa <- tempfile(pattern = "LUT_14aaG4s_", tmpdir = tempdir(), fileext = "fa")
LUT.14aaG4S.seq = ShortRead(DNAStringSet(LUT.14aaG4S$Sequence), BStringSet(LUT.14aaG4S$Names))
writeFasta(LUT.14aaG4S.seq,LUT.14aaG4S.fa)

LUT.14aaA5.fa <- tempfile(pattern = "LUT_14aaA5_", tmpdir = tempdir(), fileext = "fa")
LUT.14aaA5.seq = ShortRead(DNAStringSet(LUT.14aaA5$Sequence), BStringSet(LUT.14aaA5$Names))
writeFasta(LUT.14aaA5.seq,LUT.14aaA5.fa)

LUT.22aa.fa <- tempfile(pattern = "LUT_14aaA5_", tmpdir = tempdir(), fileext = "fa")
LUT.22aa.seq = ShortRead(DNAStringSet(LUT.22aa$Sequence), BStringSet(LUT.22aa$Names))
writeFasta(LUT.22aa.seq,LUT.22aa.fa)
```

Build Bowtie index

```
seqs.original <- readFasta("input/DNA-lib_RetrogradeTransport.fasta")

seqs.AA <- Biostrings::translate(sread(seqs.original), genetic.code=GENETIC_CODE,
                                 if.fuzzy.codon="error")

source("functions/AAtoDNA.R")
seqs.optimized = ShortRead(DNAStringSet(sapply(seqs.AA, function(x) AAtoDNA(x, species="hsa"))),
                           BStringSet(gsub("([ ])", "_", ShortRead::id(seqs.original))))

bowtie.fasta <- tempfile(pattern = "bowtie_", tmpdir = tempdir(), fileext = ".fa")

writeFasta(seqs.optimized,bowtie.fasta)

bowtie.idx <- tempfile(pattern = "IDX_bowtie_", tmpdir = tempdir(), fileext = "")
```

```
sys.out <-  system(paste("bowtie2-build",bowtie.fasta,bowtie.idx, "2>&1",  sep = " "),
                   intern = TRUE, ignore.stdout = FALSE)
```

Align fragments to reference

Align 14aa sequences

```
name.bowtie <- tempfile(pattern = "bowtie_", tmpdir = tempdir(), fileext = "")

sys.out <-  system(paste("bowtie2 --non-deterministic --threads ",detectCores(),
                         " --local --score-min 'C,0,-1' -f -a",
                         " -x ", bowtie.idx, " -U ", LUT.14aa.fa, " -S ",
                         name.bowtie, ".sam 2>&1",  sep = ""),
                   intern = TRUE, ignore.stdout = FALSE)


sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("Bowtie 2 alignment to library")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[1:lengthOut,], format = "markdown")
```

Bowtie 2 alignment to library
44708 reads; of these:
44708 (100.00%) were unpaired; of these:
0 (0.00%) aligned 0 times
34582 (77.35%) aligned exactly 1 time
10126 (22.65%) aligned >1 times
100.00% overall alignment rate

```
system(paste("samtools view -@ ",detectCores()," -Sb ", name.bowtie, ".sam > ",
             name.bowtie, ".bam",  sep = ""))
system(paste("samtools sort -@ ",detectCores()," ", name.bowtie, ".bam ",
             name.bowtie, "_sort",  sep = ""))

frag14aa.ranges <- readGAlignments(paste(name.bowtie, "_sort.bam", sep = ""), use.names=TRUE)
length(names(frag14aa.ranges))
```

```
[1] 61314
```

```
length(unique(names(frag14aa.ranges)))
```

```
[1] 44708
```

```
length(unique(LUT.14aa$Sequence))
```

```
[1] 44708
```

Align 14aaG4S sequences

```
name.bowtie <- tempfile(pattern = "bowtie_", tmpdir = tempdir(), fileext = "")

sys.out <-  system(paste("bowtie2 --non-deterministic --threads ",detectCores(),
                         " --local --score-min 'C,0,-1' -f -a",
                         " -x ", bowtie.idx, " -U ", LUT.14aaG4S.fa, " -S ",
                         name.bowtie, ".sam 2>&1",  sep = ""),
                   intern = TRUE, ignore.stdout = FALSE)

sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("Bowtie 2 alignment to library")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[1:lengthOut,], format = "markdown")
```

Bowtie 2 alignment to library
15796 reads; of these:
15796 (100.00%) were unpaired; of these:
0 (0.00%) aligned 0 times
11498 (72.79%) aligned exactly 1 time
4298 (27.21%) aligned >1 times
100.00% overall alignment rate

```
system(paste("samtools view -@ ",detectCores()," -Sb ", name.bowtie, ".sam > ",
             name.bowtie, ".bam",  sep = ""))
system(paste("samtools sort -@ ",detectCores()," ", name.bowtie, ".bam ",
             name.bowtie, "_sort",  sep = ""))

frag14aaG4S.ranges <- readGAlignments(paste(name.bowtie, "_sort.bam", sep = ""), use.names=TRUE)
length(names(frag14aaG4S.ranges))
```

```
[1] 22866
```

```
length(unique(names(frag14aaG4S.ranges)))
```

```
[1] 15796
```

```
length(unique(LUT.14aaG4S$Sequence))
```

```
[1] 15796
```

Align 14aaA5 sequences

```
name.bowtie <- tempfile(pattern = "bowtie_", tmpdir = tempdir(), fileext = "")

sys.out <-  system(paste("bowtie2 --non-deterministic --threads ",detectCores(),
                         " --local --score-min 'C,0,-1' -f -a",
                         " -x ", bowtie.idx, " -U ", LUT.14aaA5.fa, " -S ",
```

```
                        name.bowtie, ".sam 2>&1",  sep = ""),
                  intern = TRUE, ignore.stdout = FALSE)

sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("Bowtie 2 alignment to library")
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[1:lengthOut,], format = "markdown")
```

Bowtie 2 alignment to library
15796 reads; of these:
15796 (100.00%) were unpaired; of these:
0 (0.00%) aligned 0 times
11498 (72.79%) aligned exactly 1 time
4298 (27.21%) aligned >1 times
100.00% overall alignment rate

```
system(paste("samtools view -@ ",detectCores()," -Sb ", name.bowtie, ".sam > ",
             name.bowtie, ".bam",  sep = ""))
system(paste("samtools sort -@ ",detectCores()," ", name.bowtie, ".bam ",
             name.bowtie, "_sort",  sep = ""))

frag14aaA5.ranges <- readGAlignments(paste(name.bowtie, "_sort.bam", sep = ""), use.names=TRUE)
length(names(frag14aaA5.ranges))
```

```
[1] 22866
```

```
length(unique(names(frag14aaA5.ranges)))
```

```
[1] 15796
```

```
length(unique(LUT.14aaA5$Sequence))
```

```
[1] 15796
```

Align 22aa sequences

```
name.bowtie <- tempfile(pattern = "bowtie_", tmpdir = tempdir(), fileext = "")

sys.out <-  system(paste("bowtie2 --non-deterministic --threads ",detectCores(),
                         " --local --score-min 'C,0,-1' -f -a",
                         " -x ", bowtie.idx, " -U ",LUT.22aa.fa," -S ",
                         name.bowtie, ".sam 2>&1",  sep = ""),
                   intern = TRUE, ignore.stdout = FALSE)

sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("Bowtie 2 alignment to library")
```

```
invisible(sys.out[" "] <- " ")
lengthOut <- (nrow(sys.out))
knitr::kable(sys.out[1:lengthOut,], format = "markdown")
```

Bowtie 2 alignment to library
16058 reads; of these:
16058 (100.00%) were unpaired; of these:
0 (0.00%) aligned 0 times
12384 (77.12%) aligned exactly 1 time
3674 (22.88%) aligned >1 times
100.00% overall alignment rate

```
system(paste("samtools view -@ ",detectCores()," -Sb ", name.bowtie, ".sam > ",
             name.bowtie, ".bam",  sep = ""))
system(paste("samtools sort -@ ",detectCores()," ", name.bowtie, ".bam ",
             name.bowtie, "_sort",  sep = ""))

frag22aa.ranges <- readGAlignments(paste(name.bowtie, "_sort.bam", sep = ""), use.names=TRUE)
length(names(frag22aa.ranges))
```

```
[1] 21924
```

```
length(unique(names(frag22aa.ranges)))
```

```
[1] 16058
```

```
length(unique(LUT.22aa$Sequence))
```

```
[1] 16058
```

Merge and annotate aligned sequences

```
mcols(frag14aa.ranges)$structure <- "14aa"
mcols(frag22aa.ranges)$structure <- "22aa"
mcols(frag14aaA5.ranges)$structure <- "14aaA5"
mcols(frag14aaG4S.ranges)$structure <- "14aaG4S"
allFragments.ranges <- append(frag14aa.ranges,frag22aa.ranges)
allFragments.ranges <- append(allFragments.ranges,frag14aaA5.ranges)
allFragments.ranges <- append(allFragments.ranges,frag14aaG4S.ranges)

save(allFragments.ranges, file="data/alignedLibraries.rda")

devtools::session_info()
```

```
Session info -------------------------------------------------------------
```

```
 setting  value
 version  R version 3.2.3 (2015-12-10)
 system   x86_64, linux-gnu
 ui       X11
 language en_US:en
 collate  en_US.UTF-8
 tz       <NA>
 date     2016-01-13
```

Packages ----------

```
 package              * version date       source
 acepack                1.3-3.3 2014-11-24 CRAN (R 3.2.0)
 ade4                 * 1.7-3   2015-11-22 CRAN (R 3.2.2)
 Biobase              * 2.30.0  2015-11-26 Bioconductor
 BiocGenerics         * 0.16.1  2015-11-26 Bioconductor
 BiocParallel         * 1.4.0   2015-11-26 Bioconductor
 Biostrings           * 2.38.2  2015-11-26 Bioconductor
 bitops                 1.0-6   2013-08-17 CRAN (R 3.2.0)
 chron                  2.3-47  2015-06-24 CRAN (R 3.2.2)
 cluster                2.0.3   2015-07-21 CRAN (R 3.2.2)
 colorspace             1.2-6   2015-03-11 CRAN (R 3.2.0)
 data.table           * 1.9.6   2015-09-19 CRAN (R 3.2.2)
 devtools             * 1.9.1   2015-09-11 CRAN (R 3.2.2)
 digest                 0.6.8   2014-12-31 CRAN (R 3.2.0)
 evaluate               0.8     2015-09-18 CRAN (R 3.2.2)
 foreign                0.8-66  2015-08-19 CRAN (R 3.2.2)
 formatR                1.2.1   2015-09-18 CRAN (R 3.2.2)
 Formula              * 1.2-1   2015-04-07 CRAN (R 3.2.0)
 futile.logger          1.4.1   2015-04-20 CRAN (R 3.2.0)
 futile.options         1.0.0   2010-04-06 CRAN (R 3.2.0)
 GeneGA               * 1.20.0  2015-11-26 Bioconductor
 GenomeInfoDb         * 1.6.1   2015-11-26 Bioconductor
 GenomicAlignments    * 1.6.1   2015-11-26 Bioconductor
 GenomicRanges        * 1.22.1  2015-11-26 Bioconductor
 ggplot2              * 1.0.1   2015-03-17 CRAN (R 3.2.0)
 gridExtra              2.0.0   2015-07-14 CRAN (R 3.2.2)
 gtable                 0.1.2   2012-12-05 CRAN (R 3.2.0)
 hash                 * 2.2.6   2013-02-21 CRAN (R 3.2.0)
 highr                  0.5.1   2015-09-18 CRAN (R 3.2.2)
 Hmisc                * 3.17-0  2015-09-21 CRAN (R 3.2.2)
 htmltools              0.2.6   2014-09-08 CRAN (R 3.2.0)
 hwriter                1.3.2   2014-09-10 CRAN (R 3.2.0)
 IRanges              * 2.4.4   2015-11-26 Bioconductor
 knitr                * 1.11    2015-08-14 CRAN (R 3.2.2)
 lambda.r               1.1.7   2015-03-20 CRAN (R 3.2.0)
 lattice              * 0.20-33 2015-07-14 CRAN (R 3.2.2)
 latticeExtra           0.6-26  2013-08-15 CRAN (R 3.2.0)
 magrittr               1.5     2014-11-22 CRAN (R 3.2.2)
 MASS                   7.3-45  2015-11-10 CRAN (R 3.2.2)
 memoise                0.2.1   2014-04-22 CRAN (R 3.2.2)
 munsell                0.4.2   2013-07-11 CRAN (R 3.2.0)
 nnet                   7.3-11  2015-08-30 CRAN (R 3.2.2)
 plyr                   1.8.3   2015-06-12 CRAN (R 3.2.2)
 proto                  0.3-10  2012-12-22 CRAN (R 3.2.0)
 RColorBrewer           1.1-2   2014-12-07 CRAN (R 3.2.0)
 Rcpp                   0.12.2  2015-11-15 CRAN (R 3.2.2)
```

```
reshape2               1.4.1   2014-12-06 CRAN (R 3.2.0)
rmarkdown              0.8.1   2015-10-10 CRAN (R 3.2.2)
rpart                  4.1-10  2015-06-29 CRAN (R 3.2.2)
Rsamtools            * 1.22.0  2015-11-26 Bioconductor
S4Vectors            * 0.8.3   2015-11-26 Bioconductor
scales                 0.3.0   2015-08-25 CRAN (R 3.2.2)
seqinr               * 3.1-3   2014-12-17 CRAN (R 3.2.0)
ShortRead            * 1.28.0  2015-11-26 Bioconductor
stringi                1.0-1   2015-10-22 CRAN (R 3.2.2)
stringr                1.0.0   2015-04-30 CRAN (R 3.2.2)
SummarizedExperiment * 1.0.1   2015-11-26 Bioconductor
survival             * 2.38-3  2015-07-02 CRAN (R 3.2.2)
XVector              * 0.10.0  2015-11-26 Bioconductor
yaml                   2.1.13  2014-06-12 CRAN (R 3.2.0)
zlibbioc               1.16.0  2015-11-26 Bioconductor
```

Barcoded plasmid library translation

Tomas Bjorklund

Wed Jan 13 10:44:38 2016

This workflow clusters every read from each unique barcode and determines the consensus fragmnt from the CustomArray and the barcode fidelity. i.e., if the barcode was monoclonal or not..

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(parallel))
suppressPackageStartupMessages(library(doParallel))
suppressPackageStartupMessages(library(data.table))
suppressPackageStartupMessages(library(BSgenome))
suppressPackageStartupMessages(library(GenomicFeatures))
suppressPackageStartupMessages(library(GenomicAlignments))
suppressPackageStartupMessages(library(GenomicRanges))
suppressPackageStartupMessages(library(biovizBase))
suppressPackageStartupMessages(library(Gviz))
suppressPackageStartupMessages(library(plyr))
suppressPackageStartupMessages(library(devtools))
suppressPackageStartupMessages(library(Hmisc))
suppressPackageStartupMessages(library(matrixStats))
suppressPackageStartupMessages(library(stringdist))
suppressPackageStartupMessages(library(scales))

strt1<-Sys.time()

load("data/LUTdna.rda")

fragments.file <- "data/fragments_AAVlibrary_complete.fastq.gz"
barcodes.file <- "data/barcodes_AAVlibrary_complete.fastq.gz"

reads.trim <- readFastq(fragments.file)
reads.BC <- readFastq(barcodes.file)
```

Make CustomArray reference index for Blast

```
LUT.fa <- tempfile(pattern = "LUT_14aa_", tmpdir = tempdir(), fileext = ".fa")
LUT.seq = ShortRead(DNAStringSet(LUT.dna$Sequence), BStringSet(1:length(LUT.dna$Names)))
writeFasta(LUT.seq,LUT.fa)
```

Save unique fragments as fasta file

```
unique.reads <- unique(sread(reads.trim))
```

Select subset

```
#unique.reads <- unique.reads[sample(length(unique.reads), 50000)]

unique.reads <- ShortRead(DNAStringSet(unique.reads), BStringSet(1:length(unique.reads)))
fragments.unique.fa <- tempfile(pattern = "FragUnique_", tmpdir = tempdir(), fileext = ".fa")
writeFasta(unique.reads,fragments.unique.fa)
```

Align against the library using blast

```
blast.db <- tempfile(pattern = "blastDB_", tmpdir = tempdir(), fileext = ".db")
blast.out <- tempfile(pattern = "blastOut_", tmpdir = tempdir(), fileext = ".txt")

sys.out <-  system(paste("makeblastdb -in ", LUT.fa,
                         " -out ",blast.db," -dbtype nucl -title LUT -parse_seqids 2>&1",  sep = ""),
                   intern = TRUE, ignore.stdout = FALSE)

sys.out <- as.data.frame(sys.out)

colnames(sys.out) <- c("blastn database generation")
invisible(sys.out[" "] <- " ")
knitr::kable(sys.out[1:(nrow(sys.out)),], format = "markdown")
```

blastn database generation
Building a new DB, current time: 01/13/2016 10:46:12
New DB name: /tmp/RtmpoKLYWb/blastDB_1e425fd88164.db
New DB title: LUT
Sequence type: Nucleotide
Keep Linkouts: T
Keep MBits: T
Maximum file size: 1000000000B
Adding sequences from FASTA; added 92358 sequences in 2.99395 seconds.

```
sys.out <-  system(paste("export SHELL=/bin/sh; cat ",fragments.unique.fa," | parallel --block ",
                         floor(length(unique.reads)/detectCores()),
                         " --recstart '>' --pipe blastn -max_target_seqs 10 -word_size 7",
                         " -num_threads 1 -outfmt 10 -db ", blast.db,
                         " -query - > ", blast.out, " 2>&1",  sep = ""),
                   intern = TRUE, ignore.stdout = FALSE) # -word_size 7

table.blastn <- data.table(read.table(blast.out, header = FALSE, skip = 0, sep=";",
                                      stringsAsFactors = FALSE, fill=FALSE) , keep.rownames=FALSE, key="V1")

system(paste("mv", blast.out, "./data/blastOutput.csv", sep=" "))
```

```
if (length(grep("Warning",table.blastn$V1)) != 0) {
  warnings.out <- unique(table.blastn[grep("Warning",table.blastn$V1),])
  table.blastn <- table.blastn[-grep("Warning",table.blastn$V1),]
  setnames(warnings.out,"V1", c("blastn Warnings"))
  knitr::kable(warnings.out[1:(nrow(warnings.out)),], format = "markdown")
}
```

|blastn Warnings | |:———| |Warning: lcl|Query_1439 7994: Warning: Could not calculate ungapped Karlin-Altschul parameters due to an invalid query sequence or its translation. Please verify the query sequence(s) and/or filtering options |

```
table.blastn[,c("Reads","LUTnr","identity","alignmentLength","mismatches",
                "gapOpens", "q_start", "q_end", "s_start", "s_end",
                "evalue","bitScore") := tstrsplit(V1,",",fixed=TRUE),]

table.blastn[,c("V1","identity","alignmentLength","gapOpens", "q_start",
                "q_end", "s_start", "s_end", "evalue"):=NULL]

table.blastn[,Reads:= as.character(sread(unique.reads)[as.integer(Reads)])]
table.blastn[,bitScore:= as.numeric(bitScore)]
table.blastn[,mismatches:= as.numeric(mismatches)]
setkeyv(table.blastn,c("Reads","LUTnr"))
setorder(table.blastn,Reads,LUTnr,-bitScore) #This makes sure that a fragment is only aligned once to the r
table.blastn <- unique(table.blastn)


table.blastn.topHit <- table.blastn[table.blastn[, .I[which.max(bitScore)], by="Reads"]$V1] # Select only r

full.table <- data.table(Reads=as.character(sread(reads.trim)),
                         BC=as.character(sread(reads.BC)),
                         key="Reads")
all.reads <- nrow(full.table)

full.table <- full.table[table.blastn.topHit,nomatch=0] # Merge reads with the top hit alignment

print(paste("Alignment percentage:", percent(nrow(full.table)/all.reads)))
```

```
## [1] "Alignment percentage: 99.6%"
```

Starcode based barcode reduction

```
out.name.BC.star <- tempfile(pattern = "BCsc_", tmpdir = tempdir(), fileext = ".txt")

system(paste("gunzip -c ",barcodes.file," | starcode -t ",detectCores()-1," --print-clusters -d",
             1," -r5 -q -o ", out.name.BC.star, " 2>&1", sep = ""),
       intern = TRUE, ignore.stdout = FALSE)
```

```
## character(0)
```

```
table.BC.sc <- data.table(read.table(out.name.BC.star, header = FALSE, row.names = 1, skip = 0, sep="\t",
                                     stringsAsFactors = FALSE, fill=FALSE),keep.rownames=TRUE, key="rn") #,
```

```
table.BC.sc[,V2 := NULL]

table.BC.sc <- table.BC.sc[, strsplit(as.character(V3),",",fixed=TRUE), by=rn]

SC.droppedBC <- length(unique(sread(reads.BC))) - length(unique(table.BC.sc$V1) %in% unique(sread(reads.BC)))
print(paste("Dropped BCs in Starcode:", SC.droppedBC))
```

```
## [1] "Dropped BCs in Starcode: 16603"
```

```
#rm(reads.BC,reads.trim)

setnames(table.BC.sc,c("V1","rn"),c("BC","scBC"))
```

Replacing barcodes with Starcode reduced versions

```
setkey(full.table,BC)
setkey(table.BC.sc,BC)
full.table <- full.table[table.BC.sc,nomatch=0]
#full.table <- merge(full.table,table.BC.sc, by="BC", all = FALSE, all.x = FALSE)
#rm(table.BC.sc)

setnames(full.table,c("BC","scBC"),c("oldBC","BC"))

setkey(full.table,BC)

RetainedBC <- length(unique(full.table$oldBC))
scBC <- length(unique(full.table$BC))
print(paste("Original unique barcodes:", RetainedBC))
```

```
## [1] "Original unique barcodes: 3576777"
```

```
print(paste("SC reduced unique barcodes:", scBC))
```

```
## [1] "SC reduced unique barcodes: 3185961"
```

```
table.frag <- data.table(as.data.frame((rev(sort(table(full.table$oldBC))))[1:10]), keep.rownames=TRUE)
setnames(table.frag, colnames(table.frag), c("Original BC", "Count"))
knitr::kable(table.frag, format = "markdown")
```

Original BC	Count
GTGTCCATCTGGACGCGTAG	165
CATGCATGGATGACTTATGT	137
GATGGTATATATGCGGATGG	133
GTTTAAAGCAATATGCACAC	118
AATCGCTGGATGGTTTATGG	118
GCGCAATCGTGGGAACGCAC	108
GTACATTGCTGTGAGCCTGC	101

Original BC	Count
GTGCCTTTATTGGCGGCATT	99
GCGGGCGGGTGGAATGGCGT	99
GCGTATGTGCAGGCTCATTG	97

```
table.frag <- data.table(as.data.frame((rev(sort(table(full.table$BC))))[1:10]), keep.rownames=TRUE)
setnames(table.frag, colnames(table.frag), c("SC reduced BC", "Count"))
knitr::kable(table.frag, format = "markdown")
```

SC reduced BC	Count
GTGTCCATCTGGACGCGTAG	173
GATGGTATATATGCGGATGG	151
CATGCATGGATGACTTATGT	151
AATCGCTGGATGGTTTATGG	125
GTTTAAAGCAATATGCACAC	122
GCGCAATCGTGGGAACGCAC	119
GCGGGCGGGTGGAATGGCGT	117
GTACATTGCTGTGAGCCTGC	110
GCGTATGTGCAGGCTCATTG	105
CTGCGCACGCTTGTACGCGG	103

```
invisible(full.table[,oldBC:=NULL])
```

Splitting reads into single-read and multi-read barcodes

```
full.table <- full.table[order(full.table$BC),]
full.table[,mismatches:= as.numeric(mismatches)]

temp.table.single <- full.table[full.table[, .I[.N == 1], by="BC"]$V1]
temp.table.multi <- full.table[full.table[, .I[.N > 1], by="BC"]$V1]

temp.table.single[,c("mCount","tCount"):=1]
temp.table.single$Mode <- "Amb"
setkeyv(temp.table.multi,c("BC","LUTnr"))


temp.table.multi[,c("bitScore","mismatches" ,"tCount"):= list(mean(bitScore),
                                                              median(mismatches), .N),
                 by=key(temp.table.multi)]

temp.table.multi$Mode <- "Def"
temp.table.multi <- unique(temp.table.multi)

print("Utilized reads.......")
```

```
## [1] "Utilized reads......."
```

```
print(nrow(full.table))
```

```
## [1] 10638907
```

```
print("Whereof single reads.......")
```

```
## [1] "Whereof single reads......."
```

```
print(nrow(temp.table.single))
```

```
## [1] 1351591
```

Splitting multi-read barcodes into clean and chimeric

```
setkeyv(temp.table.multi,"BC")

temp.table.multi.clean <- temp.table.multi[temp.table.multi[, .I[.N == 1], by="BC"]$V1]
temp.table.multi <- temp.table.multi[temp.table.multi[, .I[.N > 1], by="BC"]$V1]


temp.table.multi.clean[,mCount:=tCount]

print("Clean multi-read barcodes.......")
```

```
## [1] "Clean multi-read barcodes......."
```

```
print(nrow(temp.table.multi.clean))
```

```
## [1] 454777
```

```
print("Chimeric multi-read barcodes.......")
```

```
## [1] "Chimeric multi-read barcodes......."
```

```
print(length(unique(temp.table.multi$BC)))
```

```
## [1] 1379593
```

Calculate consensus alignment of chimeric barcodes

```
setkey(temp.table.multi,"BC")
temp.table.multi[, "mCount":=tCount]
temp.table.multi[, "tCount":=sum(tCount), by="BC"]

setkey(temp.table.multi,"Reads")
```

```
temp.table.multi[,c("LUTnr","bitScore","mismatches"):=NULL]
setkey(table.blastn,"Reads")
temp.table.multi <- temp.table.multi[table.blastn, nomatch=0, allow.cartesian=TRUE]

setkeyv(temp.table.multi,c("BC","LUTnr"))
temp.table.multi[,c("bitScore","mismatches" ,"mCount"):= list(max(bitScore),
                                                              median(mismatches),
                                                              sum(mCount)), by=key(temp.table.multi)]
temp.table.multi <- unique(temp.table.multi)

setkeyv(temp.table.multi,"BC")
temp.table.multi <- temp.table.multi[temp.table.multi[, .I[mCount == max(mCount)],
                                                      by=key(temp.table.multi)]$V1]
# Select only rows with the highest mCount

temp.table.multi <- temp.table.multi[temp.table.multi[, .I[which.max(bitScore)],
                                                      by=key(temp.table.multi)]$V1]
# Select only rows with the highest bitScore

temp.table.multi[temp.table.multi$mCount==1]$Mode <- "Amb"

print(paste("Number of barcodes with false mCount:",
            nrow(temp.table.multi[mCount > tCount])))
```

```
## [1] "Number of barcodes with false mCount: 87365"
```

```
temp.table.multi.consensus <- rbind(temp.table.multi, temp.table.multi.clean)

print(paste("Total number of definitive Barcodes:",
            length(grep("Def", temp.table.multi.consensus$Mode))))
```

```
## [1] "Total number of definitive Barcodes: 1642247"
```

```
print(paste("Total number of ambiguous Barcodes:",
            length(grep("Amb", temp.table.multi.consensus$Mode))))
```

```
## [1] "Total number of ambiguous Barcodes: 192123"
```

```
print(paste("Total number of single-read Barcodes:",
            nrow(temp.table.single)))
```

```
## [1] "Total number of single-read Barcodes: 1351591"
```

```
output.Table <- rbind(temp.table.multi.consensus,temp.table.single)
save(output.Table, file="data/multipleContfragmentsComplete.rda")

print("Total analysis time:")
```

```
## [1] "Total analysis time:"
```

```
print(Sys.time()-strt1)
```

```
## Time difference of 2.187571 hours
```

```
devtools::session_info()
```

```
## Session info -------------------------------------------------------------

##  setting  value
##  version  R version 3.2.3 (2015-12-10)
##  system   x86_64, linux-gnu
##  ui       X11
##  language en_US:en
##  collate  en_US.UTF-8
##  tz       <NA>
##  date     2016-01-13

## Packages -----------------------------------------------------------------

##  package              * version  date       source
##  acepack                1.3-3.3  2014-11-24 CRAN (R 3.2.0)
##  AnnotationDbi        * 1.32.0   2015-11-26 Bioconductor
##  Biobase              * 2.30.0   2015-11-26 Bioconductor
##  BiocGenerics         * 0.16.1   2015-11-26 Bioconductor
##  BiocParallel         * 1.4.0    2015-11-26 Bioconductor
##  biomaRt                2.26.1   2015-11-26 Bioconductor
##  Biostrings           * 2.38.2   2015-11-26 Bioconductor
##  biovizBase           * 1.18.0   2015-11-26 Bioconductor
##  bitops                 1.0-6    2013-08-17 CRAN (R 3.2.0)
##  BSgenome             * 1.38.0   2015-11-26 Bioconductor
##  chron                  2.3-47   2015-06-24 CRAN (R 3.2.2)
##  cluster                2.0.3    2015-07-21 CRAN (R 3.2.2)
##  codetools              0.2-14   2015-07-15 CRAN (R 3.2.2)
##  colorspace             1.2-6    2015-03-11 CRAN (R 3.2.0)
##  data.table           * 1.9.6    2015-09-19 CRAN (R 3.2.2)
##  DBI                    0.3.1    2014-09-24 CRAN (R 3.2.0)
##  devtools             * 1.9.1    2015-09-11 CRAN (R 3.2.2)
##  dichromat              2.0-0    2013-01-24 CRAN (R 3.2.0)
##  digest                 0.6.8    2014-12-31 CRAN (R 3.2.0)
##  doParallel           * 1.0.10   2015-10-14 CRAN (R 3.2.2)
##  evaluate               0.8      2015-09-18 CRAN (R 3.2.2)
##  foreach              * 1.4.3    2015-10-13 CRAN (R 3.2.2)
##  foreign                0.8-66   2015-08-19 CRAN (R 3.2.2)
##  formatR                1.2.1    2015-09-18 CRAN (R 3.2.2)
##  Formula              * 1.2-1    2015-04-07 CRAN (R 3.2.0)
##  futile.logger          1.4.1    2015-04-20 CRAN (R 3.2.0)
##  futile.options         1.0.0    2010-04-06 CRAN (R 3.2.0)
##  GenomeInfoDb         * 1.6.1    2015-11-26 Bioconductor
##  GenomicAlignments    * 1.6.1    2015-11-26 Bioconductor
##  GenomicFeatures      * 1.22.5   2015-11-26 Bioconductor
##  GenomicRanges        * 1.22.1   2015-11-26 Bioconductor
##  ggplot2              * 1.0.1    2015-03-17 CRAN (R 3.2.0)
##  gridExtra              2.0.0    2015-07-14 CRAN (R 3.2.2)
##  gtable                 0.1.2    2012-12-05 CRAN (R 3.2.0)
##  Gviz                 * 1.14.0   2015-11-26 Bioconductor
##  highr                  0.5.1    2015-09-18 CRAN (R 3.2.2)
##  Hmisc                * 3.17-0   2015-09-21 CRAN (R 3.2.2)
##  htmltools              0.2.6    2014-09-08 CRAN (R 3.2.0)
##  hwriter                1.3.2    2014-09-10 CRAN (R 3.2.0)
##  IRanges              * 2.4.4    2015-11-26 Bioconductor
```

```
##  iterators            * 1.0.8    2015-10-13 CRAN (R 3.2.2)
##  knitr                * 1.11     2015-08-14 CRAN (R 3.2.2)
##  lambda.r               1.1.7    2015-03-20 CRAN (R 3.2.0)
##  lattice              * 0.20-33  2015-07-14 CRAN (R 3.2.2)
##  latticeExtra           0.6-26   2013-08-15 CRAN (R 3.2.0)
##  magrittr               1.5      2014-11-22 CRAN (R 3.2.2)
##  MASS                   7.3-45   2015-11-10 CRAN (R 3.2.2)
##  matrixStats          * 0.15.0   2015-10-27 CRAN (R 3.2.2)
##  memoise                0.2.1    2014-04-22 CRAN (R 3.2.2)
##  munsell                0.4.2    2013-07-11 CRAN (R 3.2.0)
##  nnet                   7.3-11   2015-08-30 CRAN (R 3.2.2)
##  plyr                 * 1.8.3    2015-06-12 CRAN (R 3.2.2)
##  proto                  0.3-10   2012-12-22 CRAN (R 3.2.0)
##  RColorBrewer           1.1-2    2014-12-07 CRAN (R 3.2.0)
##  Rcpp                   0.12.2   2015-11-15 CRAN (R 3.2.2)
##  RCurl                  1.95-4.7 2015-06-30 CRAN (R 3.2.2)
##  reshape2               1.4.1    2014-12-06 CRAN (R 3.2.0)
##  rmarkdown              0.8.1    2015-10-10 CRAN (R 3.2.2)
##  rpart                  4.1-10   2015-06-29 CRAN (R 3.2.2)
##  Rsamtools            * 1.22.0   2015-11-26 Bioconductor
##  RSQLite                1.0.0    2014-10-25 CRAN (R 3.2.0)
##  rtracklayer          * 1.30.1   2015-11-26 Bioconductor
##  S4Vectors            * 0.8.3    2015-11-26 Bioconductor
##  scales               * 0.3.0    2015-08-25 CRAN (R 3.2.2)
##  ShortRead            * 1.28.0   2015-11-26 Bioconductor
##  stringdist           * 0.9.4    2015-10-26 CRAN (R 3.2.2)
##  stringi                1.0-1    2015-10-22 CRAN (R 3.2.2)
##  stringr                1.0.0    2015-04-30 CRAN (R 3.2.2)
##  SummarizedExperiment * 1.0.1    2015-11-26 Bioconductor
##  survival             * 2.38-3   2015-07-02 CRAN (R 3.2.2)
##  VariantAnnotation      1.16.3   2015-11-26 Bioconductor
##  XML                    3.98-1.3 2015-06-30 CRAN (R 3.2.2)
##  XVector              * 0.10.0   2015-11-26 Bioconductor
##  yaml                   2.1.13   2014-06-12 CRAN (R 3.2.0)
##  zlibbioc               1.16.0   2015-11-26 Bioconductor
```

Library analysis output

Tomas Bjorklund

Wed Jan 13 12:56:17 2016

This workflow brings together FastQ files containing barcodes and 5'/3' ends of a suitable insert and alignmen them using Bowtie2. It also includes starcode based false barcode reduction and a MapReduce based hierarchical clustering

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(ggplot2))
suppressPackageStartupMessages(library(ggbio))
suppressPackageStartupMessages(library(beanplot))
suppressPackageStartupMessages(library(parallel))
suppressPackageStartupMessages(library(doParallel))
suppressPackageStartupMessages(library(data.table))
suppressPackageStartupMessages(library(scales)) #Gives the log2 ability to ggplot2
suppressPackageStartupMessages(library(formatR))
suppressPackageStartupMessages(library(BSgenome))
suppressPackageStartupMessages(library(Rsamtools))
suppressPackageStartupMessages(library(rtracklayer))
suppressPackageStartupMessages(library(GenomicFeatures))
suppressPackageStartupMessages(library(GenomicAlignments))
suppressPackageStartupMessages(library(GenomicRanges))
suppressPackageStartupMessages(library(biovizBase))
suppressPackageStartupMessages(library(Gviz))
suppressPackageStartupMessages(library(plyr))
suppressPackageStartupMessages(library(devtools))
strt <- Sys.time()
load("data/multipleContfragmentsComplete.rda")
load("data/alignedLibraries.rda")
load("data/LUTdna.rda")

load.list <- read.table("input/loadlist.txt", header = FALSE, skip = 0, sep="\t",
                        stringsAsFactors = FALSE, fill=TRUE)

dataDir <- "seqFiles/RNA"
colnames(load.list) <- c("Name", "BaseName","GroupName")

log.table <- data.table(Name="Name",
                        Reads=NA,
                        Purity=NA,
                        BCs=NA,
                        SCdroppedBC=NA,
                        allBCs=NA,
                        scBCs=NA)

analyzeTissue <- function(indexNr) {
#indexNr <- 15

  name <- unlist(strsplit(load.list$BaseName[indexNr],"/"))
  name <- name[!is.na(name)]
  if (length(name)==2){
    in.files <- list.files(paste(gsub("([\\])", "", dataDir),name[1],sep="/"),
                           pattern=paste(name[2],"*", sep=""), full.names=TRUE)
  } else {
```

```
    in.files <- list.files(gsub("([\\])", "", dataDir),
                           pattern=paste(name[1],"*", sep=""), full.names=TRUE)
  }
  in.files.P5 <-in.files[grep("R1",in.files)]
  in.files.P7 <- in.files[grep("R2",in.files)]

in.name.P5 <- tempfile(pattern = "P5_", tmpdir = tempdir(), fileext = ".fastq.gz")
in.name.P7 <- tempfile(pattern = "P7_", tmpdir = tempdir(), fileext = ".fastq.gz")
system(paste("cat '", paste(as.character(in.files.P5), collapse="' '"), "' > ",
             in.name.P5, " 2>&1", sep = ""), intern = TRUE, ignore.stdout = FALSE)

system(paste("cat '", paste(as.character(in.files.P7), collapse="' '"), "' > ",
             in.name.P7, " 2>&1", sep = ""), intern = TRUE, ignore.stdout = FALSE)

log.table$Name <- load.list$Name[indexNr]
name.out <- log.table$Name

# Selection of real amplicons
# ===========================

out.name.P5 <- tempfile(pattern = "P5_", tmpdir = tempdir(), fileext = ".fastq.gz")
out.name.P7 <- tempfile(pattern = "P7_", tmpdir = tempdir(), fileext = ".fastq.gz")
command.args <- paste("-Xmx12g overwrite=true k=10 rcomp=f skipr1=t qhdist=0 maskmiddle=t ",
                      "hammingdistance=1 findbestmatch=t ordered=t threads=",detectCores(),
                      " in=", in.name.P5,
                      " in2=", in.name.P7,
                      " outm=", out.name.P5,
                      " outm2=", out.name.P7,
                      " fliteral=", "CGCCACAACATCGAGGACGGCAGCGTG", sep = "")

sys.out <- system2(path.expand("~/bbmap/bbduk2.sh"), args=command.args, stdout=TRUE, stderr=TRUE)
log.table$Purity <- strsplit(sys.out[grep("Contaminants",sys.out)],split = "\t")[[1]][2]

in.name.P5 <- out.name.P5
in.name.P7 <- out.name.P7

log.table$Reads <- as.integer(system(paste("gunzip -c ",shQuote(gsub("([\\])", "", in.name.P5)),
                                           " | echo $((`wc -l`/4)) 2>&1", sep = ""), intern = TRUE,
                                     ignore.stdout = FALSE)) #Stores the read count utilized


# Extraction of barcodes
# ======================

out.name.BC <- tempfile(pattern = "BC_", tmpdir = tempdir(), fileext = ".fastq.gz")

sys.out <- system(paste("~/bbmap/bbduk2.sh overwrite=true k=12 mink=12 hammingdistance=2 ",
                        "findbestmatch=t trd=t rcomp=f skipr2=t findbestmatch=f qhdist=0 ",
                        "minavgquality=0 ordered=t maxns=0 minlength=18 maxlength=22 threads=",
                        detectCores()," in=", shQuote(in.name.P5), " out=", out.name.BC,
                        " lliteral=", "GGCCTAGCGGCCGCTTTACTT", " rliteral=", "ATAACTTCGTATA",
                        " 2>&1", sep = ""), intern = TRUE, ignore.stdout = FALSE)

log.table$BCs <- strsplit(sys.out[grep("Result:",sys.out)],split = "\t")[[1]][2]

reads.BC <- readFastq(out.name.BC)
barcodeTable <- data.table(ID=as.character(ShortRead::id(reads.BC)),
```

```
                              BC=as.character(sread(reads.BC)), key="BC")

# Starcode based barcode reduction
# ============================

out.name.BC.star <- tempfile(pattern = "BCsc_", tmpdir = tempdir(), fileext = ".txt")

system(paste("gunzip -c ",out.name.BC," | starcode -t ",detectCores()-1," --print-clusters -d",
             1," -r5 -q -o ", out.name.BC.star, " 2>&1", sep = ""),
       intern = TRUE, ignore.stdout = FALSE)

table.BC.sc <- data.table(read.table(out.name.BC.star, header = FALSE, row.names = 1, skip = 0, sep="\t",
                                     stringsAsFactors = FALSE, fill=FALSE),keep.rownames=TRUE, key="rn")
table.BC.sc[,V2 := NULL]

table.BC.sc <- table.BC.sc[, strsplit(as.character(V3),",",fixed=TRUE), by=rn]

log.table$SCdroppedBC <- length(unique(sread(reads.BC))) - length(unique(table.BC.sc$V1) %in% unique(sread(r


setnames(table.BC.sc,c("V1","rn"),c("BC","scBC"))

# Replacing barcodes with Starcode reduced versions
# ============================


setkey(table.BC.sc,BC)

barcodeTable <- barcodeTable[table.BC.sc,nomatch=0]

setnames(barcodeTable,c("BC","scBC"),c("oldBC","BC"))

setkey(barcodeTable,BC)

log.table$allBCs <- length(unique(barcodeTable$oldBC))
log.table$scBCs <- length(unique(barcodeTable$BC))

invisible(barcodeTable[,oldBC:=NULL])
setkey(output.Table,"BC")

BCcount <- rev(sort(table(barcodeTable$BC)))

foundFrags <- output.Table[match(names(BCcount), output.Table$BC),]
foundFrags$RNAcount <- as.integer(BCcount)
foundFrags <- na.omit(foundFrags)
foundFrags$fragment <- LUT.dna$Sequence[as.integer(foundFrags$LUTnr)]

matchRange <- function(idxFrag) {
  #idxFrag <- 23
  matchRanges <- which(names(allFragments.ranges) == foundFrags$fragment[idxFrag])
  return(cbind(matchRanges,idxFrag))
}
match.ranges.list <- mclapply(1:nrow(foundFrags), matchRange, mc.preschedule = TRUE,
                              mc.cores = detectCores())
match.ranges <- do.call(rbind, match.ranges.list)
foundFragments.ranges <- allFragments.ranges[match.ranges[,1]]
if (ncol(match.ranges) >= 2) {
```

```
foundFrags <- foundFrags[match.ranges[,"idxFrag"],]
foundFrags[,c("Reads","fragment"):=NULL]
mcols(foundFragments.ranges) <- c(mcols(foundFragments.ranges),
                                  foundFrags[match.ranges[,"idxFrag"],])
o = order(-mcols(foundFragments.ranges)$RNAcount)
foundFragments.ranges <- foundFragments.ranges[o]
saveRDS(foundFragments.ranges, file=paste("output/","found.",name.out,".rds", sep=""),
        compress = TRUE)
}
return(log.table)
}
```

Analysis summary

```
all.logs <- lapply(1:nrow(load.list), analyzeTissue)
all.logs <- rbindlist(all.logs, use.names=FALSE )
knitr::kable(all.logs, format = "markdown")
```

Name	Reads	Purity	BCs	SCdroppedBC	allBCs
RatNr18_4wks_1000x_Sc-12	1556292	3112584 reads (99.48%)	813311 reads (52.26%)	11	3586
RatNr25_4wks_Untreat_Mu-13	1968380	3936760 reads (99.28%)	277622 reads (14.10%)	3	1200
RatNr25_4wks_Untreat_Sc-14	2120478	4240956 reads (99.18%)	200004 reads (9.43%)	0	1305
RatNr2_4wks_100x_Ctx-1	1955085	3910170 reads (99.25%)	591146 reads (30.24%)	3	5607
RatNr2_4wks_100x_SN-2	1921383	3842766 reads (99.39%)	1210727 reads (63.01%)	10	18161
RatNr2_4wks_100x_Str-3	2101122	4202244 reads (100.00%)	1563614 reads (74.42%)	24	84742
RatNr2_4wks_100x_Th-4	2177483	4354966 reads (95.47%)	752026 reads (34.54%)	0	16068
RatNr5_4wks_100x_Mu-5	2035694	4071388 reads (99.50%)	403591 reads (19.83%)	1	6078
RatNr5_4wks_100x_Sc-6	2441451	4882902 reads (97.96%)	632745 reads (25.92%)	10	2174
RatNr13_4wks_1000x_Ctx-7	2070763	4141526 reads (99.61%)	183573 reads (8.86%)	1	1403
RatNr13_4wks_1000x_SN-8	1809233	3618466 reads (99.47%)	105370 reads (5.82%)	2	1696
RatNr13_4wks_1000x_Str-9	1693037	3386074 reads (99.32%)	1096589 reads (64.77%)	2	17281
RatNr13_4wks_1000x_Th-10	1954529	3909058 reads (99.61%)	1079929 reads (55.25%)	27	12266
RatNr18_4wks_1000x_Mu-11	2069527	4139054 reads (99.35%)	358745 reads (17.33%)	3	3191
RatNr7_100x_SN-1	1983137	3966274 reads (99.65%)	1614014 reads (81.39%)	41	24691
RatNr7_100x_Ctx-2	1994467	3988934 reads (99.60%)	1771085 reads (88.80%)	13	16108
RatNr7_100x_Str-3	2867972	5735944 reads (99.65%)	1382945 reads (48.22%)	12	30889
RatNr7_100x_Th-4	1596468	3192936 reads (99.62%)	991144 reads (62.08%)	15	60701
RatNr1_100x_SN-5	1611759	3223518 reads (99.67%)	1361550 reads (84.48%)	47	11471
RatNr1_100x_Ctx-6	1541657	3083314 reads (99.67%)	1203038 reads (78.04%)	9	11542
RatNr1_100x_Str-7	2359538	4719076 reads (99.67%)	1026288 reads (43.50%)	1	17976
RatNr1_100x_Th-8	1505088	3010176 reads (99.60%)	619506 reads (41.16%)	6	37192
RatNr8_100x_SN-9	2054931	4109862 reads (99.70%)	1215848 reads (59.17%)	80	22685
RatNr8_100x_Ctx-10	2102816	4205632 reads (99.69%)	1081123 reads (51.41%)	18	13376

Name	Reads	Purity	BCs	SCdroppedBC	allBCs
RatNr8_100x_Str-11	2105768	4211536 reads (99.69%)	856547 reads (40.68%)	9	29605
RatNr8_100x_Th-12	1623686	3247372 reads (99.64%)	612950 reads (37.75%)	9	45404
RatNr15_1000x_SN-13	1436268	2872536 reads (99.64%)	1435201 reads (99.93%)	17	4226
RatNr15_1000x_Ctx-14	1260242	2520484 reads (99.65%)	1062278 reads (84.29%)	12	6434
RatNr15_1000x_Str-15	1105966	2211932 reads (99.65%)	944716 reads (85.42%)	3	5642
RatNr15_1000x_Th-16	948187	1896374 reads (99.65%)	684628 reads (72.20%)	2	22301
RatNr21_1000x_SN-17	1115267	2230534 reads (99.75%)	1112638 reads (99.76%)	3	4489
RatNr21_1000x_Ctx-18	1201263	2402526 reads (99.73%)	788753 reads (65.66%)	2	3920
RatNr21_1000x_Str-19	1234915	2469830 reads (99.73%)	1063068 reads (86.08%)	0	7414
RatNr21_1000x_Th-20	1151549	2303098 reads (99.71%)	845867 reads (73.45%)	3	29847
RatNr19_1000x_Ctx-21	1743952	3487904 reads (99.61%)	1738647 reads (99.70%)	15	6568
RatNr19_1000x_Str-22	1738722	3477444 reads (99.68%)	1174178 reads (67.53%)	21	10753
RatNr19_1000x_Th-23	1645506	3291012 reads (99.67%)	1196277 reads (72.70%)	5	34666
RatNr20_1000x_Str-24	788088	1576176 reads (99.61%)	682035 reads (86.54%)	3	5098
RatNr20_1000x_Th-25	983263	1966526 reads (99.59%)	827836 reads (84.19%)	3	23426
Cells293Nr2_1000x_cDNA-26	1588150	3176300 reads (99.63%)	1016131 reads (63.98%)	11	7051
Cells293Nr3_100x_cDNA-27	1920390	3840780 reads (99.65%)	864987 reads (45.04%)	13	20920
primNeuronsNr6_1000x_cDNA-28	1143395	2286790 reads (99.56%)	552817 reads (48.35%)	4	6313
primNeuronsNr7_100x_cDNA-29	1652464	3304928 reads (99.66%)	942060 reads (57.01%)	13	24858
primNeuronsNr6_1000x_RNA-30	1739141	3478282 reads (99.60%)	780217 reads (44.86%)	9	6942

```
knitr::kable(all.logs, format = "markdown")
```

Name	Reads	Purity	BCs	SCdroppedBC	allBCs
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Name	Reads	Purity	BCs	SCdroppedBC	allBCs
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RatNr7_100x_Str-3	2867972	5735944 reads (99.65%)	1382945 reads (48.22%)	12	30889
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RatNr21_1000x_Th-20	1151549	2303098 reads (99.71%)	845867 reads (73.45%)	3	29847
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RatNr19_1000x_Str-22	1738722	3477444 reads (99.68%)	1174178 reads (67.53%)	21	10753
RatNr19_1000x_Th-23	1645506	3291012 reads (99.67%)	1196277 reads (72.70%)	5	34666
RatNr20_1000x_Str-24	788088	1576176 reads (99.61%)	682035 reads (86.54%)	3	5098
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primNeuronsNr7_100x_cDNA-29	1652464	3304928 reads (99.66%)	942060 reads (57.01%)	13	24858
primNeuronsNr6_1000x_RNA-30	1739141	3478282 reads (99.60%)	780217 reads (44.86%)	9	6942

```
unlink(paste(tempdir(), "/*", sep = ""), recursive = FALSE, force = FALSE) #Cleanup of temp files

print("Total execution time:")
```

```
## [1] "Total execution time:"
```

```
print(Sys.time()-strt)
```

```
## Time difference of 18.12098 mins
```

```
devtools::session_info()
```

```
## Session info -------------------------------------------------------------

##  setting  value
##  version  R version 3.2.3 (2015-12-10)
##  system   x86_64, linux-gnu
##  ui       X11
##  language en_US:en
##  collate  en_US.UTF-8
##  tz       <NA>
##  date     2016-01-13

## Packages -----------------------------------------------------------------

##  package              * version  date       source
##  acepack                1.3-3.3  2014-11-24 CRAN (R 3.2.0)
##  AnnotationDbi        * 1.32.0   2015-11-26 Bioconductor
##  beanplot             * 1.2      2014-09-19 CRAN (R 3.2.0)
##  Biobase              * 2.30.0   2015-11-26 Bioconductor
##  BiocGenerics         * 0.16.1   2015-11-26 Bioconductor
##  BiocInstaller          1.20.1   2015-11-26 Bioconductor
##  BiocParallel         * 1.4.0    2015-11-26 Bioconductor
##  biomaRt                2.26.1   2015-11-26 Bioconductor
##  Biostrings           * 2.38.2   2015-11-26 Bioconductor
##  biovizBase           * 1.18.0   2015-11-26 Bioconductor
##  bitops                 1.0-6    2013-08-17 CRAN (R 3.2.0)
##  BSgenome             * 1.38.0   2015-11-26 Bioconductor
##  chron                  2.3-47   2015-06-24 CRAN (R 3.2.2)
##  cluster                2.0.3    2015-07-21 CRAN (R 3.2.2)
##  codetools              0.2-14   2015-07-15 CRAN (R 3.2.2)
##  colorspace             1.2-6    2015-03-11 CRAN (R 3.2.0)
##  data.table           * 1.9.6    2015-09-19 CRAN (R 3.2.2)
##  DBI                    0.3.1    2014-09-24 CRAN (R 3.2.0)
##  devtools             * 1.9.1    2015-09-11 CRAN (R 3.2.2)
##  dichromat              2.0-0    2013-01-24 CRAN (R 3.2.0)
##  digest                 0.6.8    2014-12-31 CRAN (R 3.2.0)
##  doParallel           * 1.0.10   2015-10-14 CRAN (R 3.2.2)
##  evaluate               0.8      2015-09-18 CRAN (R 3.2.2)
##  foreach              * 1.4.3    2015-10-13 CRAN (R 3.2.2)
##  foreign                0.8-66   2015-08-19 CRAN (R 3.2.2)
##  formatR              * 1.2.1    2015-09-18 CRAN (R 3.2.2)
##  Formula                1.2-1    2015-04-07 CRAN (R 3.2.0)
##  futile.logger          1.4.1    2015-04-20 CRAN (R 3.2.0)
##  futile.options         1.0.0    2010-04-06 CRAN (R 3.2.0)
##  GenomeInfoDb         * 1.6.1    2015-11-26 Bioconductor
##  GenomicAlignments    * 1.6.1    2015-11-26 Bioconductor
##  GenomicFeatures      * 1.22.5   2015-11-26 Bioconductor
##  GenomicRanges        * 1.22.1   2015-11-26 Bioconductor
##  GGally                 0.5.0    2014-12-02 CRAN (R 3.2.0)
##  ggbio                * 1.18.1   2015-11-26 Bioconductor
##  ggplot2              * 1.0.1    2015-03-17 CRAN (R 3.2.0)
##  graph                  1.48.0   2015-11-26 Bioconductor
##  gridExtra              2.0.0    2015-07-14 CRAN (R 3.2.2)
##  gtable                 0.1.2    2012-12-05 CRAN (R 3.2.0)
##  Gviz                 * 1.14.0   2015-11-26 Bioconductor
```

```
##  highr                  0.5.1    2015-09-18 CRAN (R 3.2.2)
##  Hmisc                  3.17-0   2015-09-21 CRAN (R 3.2.2)
##  htmltools              0.2.6    2014-09-08 CRAN (R 3.2.0)
##  hwriter                1.3.2    2014-09-10 CRAN (R 3.2.0)
##  IRanges              * 2.4.4    2015-11-26 Bioconductor
##  iterators            * 1.0.8    2015-10-13 CRAN (R 3.2.2)
##  knitr                * 1.11     2015-08-14 CRAN (R 3.2.2)
##  lambda.r               1.1.7    2015-03-20 CRAN (R 3.2.0)
##  lattice                0.20-33  2015-07-14 CRAN (R 3.2.2)
##  latticeExtra           0.6-26   2013-08-15 CRAN (R 3.2.0)
##  magrittr               1.5      2014-11-22 CRAN (R 3.2.2)
##  MASS                   7.3-45   2015-11-10 CRAN (R 3.2.2)
##  matrixStats            0.15.0   2015-10-27 CRAN (R 3.2.2)
##  memoise                0.2.1    2014-04-22 CRAN (R 3.2.2)
##  munsell                0.4.2    2013-07-11 CRAN (R 3.2.0)
##  nnet                   7.3-11   2015-08-30 CRAN (R 3.2.2)
##  OrganismDbi            1.12.0   2015-11-26 Bioconductor
##  plyr                 * 1.8.3    2015-06-12 CRAN (R 3.2.2)
##  proto                  0.3-10   2012-12-22 CRAN (R 3.2.0)
##  RBGL                   1.46.0   2015-11-26 Bioconductor
##  RColorBrewer           1.1-2    2014-12-07 CRAN (R 3.2.0)
##  Rcpp                   0.12.2   2015-11-15 CRAN (R 3.2.2)
##  RCurl                  1.95-4.7 2015-06-30 CRAN (R 3.2.2)
##  reshape                0.8.5    2014-04-23 CRAN (R 3.2.0)
##  reshape2               1.4.1    2014-12-06 CRAN (R 3.2.0)
##  rmarkdown              0.8.1    2015-10-10 CRAN (R 3.2.2)
##  rpart                  4.1-10   2015-06-29 CRAN (R 3.2.2)
##  Rsamtools            * 1.22.0   2015-11-26 Bioconductor
##  RSQLite                1.0.0    2014-10-25 CRAN (R 3.2.0)
##  rtracklayer          * 1.30.1   2015-11-26 Bioconductor
##  S4Vectors            * 0.8.3    2015-11-26 Bioconductor
##  scales               * 0.3.0    2015-08-25 CRAN (R 3.2.2)
##  ShortRead            * 1.28.0   2015-11-26 Bioconductor
##  stringi                1.0-1    2015-10-22 CRAN (R 3.2.2)
##  stringr                1.0.0    2015-04-30 CRAN (R 3.2.2)
##  SummarizedExperiment * 1.0.1    2015-11-26 Bioconductor
##  survival               2.38-3   2015-07-02 CRAN (R 3.2.2)
##  VariantAnnotation      1.16.3   2015-11-26 Bioconductor
##  XML                    3.98-1.3 2015-06-30 CRAN (R 3.2.2)
##  XVector              * 0.10.0   2015-11-26 Bioconductor
##  yaml                   2.1.13   2014-06-12 CRAN (R 3.2.0)
##  zlibbioc               1.16.0   2015-11-26 Bioconductor
```

Generate a complete library range object

Tomas Bjorklund

Wed Jan 13 13:14:49 2016

This workflow clusters every read from each unique barcode from the library and matches them to the relevant ranges.

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(parallel))
suppressPackageStartupMessages(library(doParallel))
suppressPackageStartupMessages(library(data.table))
suppressPackageStartupMessages(library(devtools))

load("data/alignedLibraries.rda")
load("data/LUTdna.rda")
load("data/multipleContfragmentsComplete.rda")
output.Table$fragment <- LUT.dna$Sequence[as.integer(output.Table$LUTnr)]
setkey(output.Table,fragment)

range.idx <- data.table(fragment=names(allFragments.ranges),
                        idxFrag=1:length(allFragments.ranges), key="fragment")
output.Table <- output.Table[range.idx, nomatch=0, allow.cartesian=TRUE]

foundFragments.ranges <- allFragments.ranges[output.Table$idxFrag]
output.Table[,c("Reads","fragment","idxFrag"):=NULL]
mcols(foundFragments.ranges) <- c(mcols(foundFragments.ranges), output.Table)

saveRDS(foundFragments.ranges, file="output/completeLibraryRanges.rds")

devtools::session_info()
```

```
## Session info -------------------------------------------------------------

##  setting  value
##  version  R version 3.2.3 (2015-12-10)
##  system   x86_64, linux-gnu
##  ui       X11
##  language en_US:en
##  collate  en_US.UTF-8
##  tz       <NA>
##  date     2016-01-13

## Packages -----------------------------------------------------------------

##  package              * version date       source
##  Biobase              * 2.30.0  2015-11-26 Bioconductor
##  BiocGenerics         * 0.16.1  2015-11-26 Bioconductor
##  BiocParallel         * 1.4.0   2015-11-26 Bioconductor
##  Biostrings           * 2.38.2  2015-11-26 Bioconductor
##  bitops                 1.0-6   2013-08-17 CRAN (R 3.2.0)
##  chron                  2.3-47  2015-06-24 CRAN (R 3.2.2)
##  codetools              0.2-14  2015-07-15 CRAN (R 3.2.2)
##  data.table           * 1.9.6   2015-09-19 CRAN (R 3.2.2)
```

```
##  devtools             * 1.9.1   2015-09-11 CRAN (R 3.2.2)
##  digest                 0.6.8   2014-12-31 CRAN (R 3.2.0)
##  doParallel           * 1.0.10  2015-10-14 CRAN (R 3.2.2)
##  evaluate               0.8     2015-09-18 CRAN (R 3.2.2)
##  foreach              * 1.4.3   2015-10-13 CRAN (R 3.2.2)
##  futile.logger          1.4.1   2015-04-20 CRAN (R 3.2.0)
##  futile.options         1.0.0   2010-04-06 CRAN (R 3.2.0)
##  GenomeInfoDb         * 1.6.1   2015-11-26 Bioconductor
##  GenomicAlignments    * 1.6.1   2015-11-26 Bioconductor
##  GenomicRanges        * 1.22.1  2015-11-26 Bioconductor
##  htmltools              0.2.6   2014-09-08 CRAN (R 3.2.0)
##  hwriter                1.3.2   2014-09-10 CRAN (R 3.2.0)
##  IRanges              * 2.4.4   2015-11-26 Bioconductor
##  iterators            * 1.0.8   2015-10-13 CRAN (R 3.2.2)
##  knitr                * 1.11    2015-08-14 CRAN (R 3.2.2)
##  lambda.r               1.1.7   2015-03-20 CRAN (R 3.2.0)
##  lattice                0.20-33 2015-07-14 CRAN (R 3.2.2)
##  latticeExtra           0.6-26  2013-08-15 CRAN (R 3.2.0)
##  magrittr               1.5     2014-11-22 CRAN (R 3.2.2)
##  memoise                0.2.1   2014-04-22 CRAN (R 3.2.2)
##  RColorBrewer           1.1-2   2014-12-07 CRAN (R 3.2.0)
##  rmarkdown              0.8.1   2015-10-10 CRAN (R 3.2.2)
##  Rsamtools            * 1.22.0  2015-11-26 Bioconductor
##  S4Vectors            * 0.8.3   2015-11-26 Bioconductor
##  ShortRead            * 1.28.0  2015-11-26 Bioconductor
##  stringi                1.0-1   2015-10-22 CRAN (R 3.2.2)
##  stringr                1.0.0   2015-04-30 CRAN (R 3.2.2)
##  SummarizedExperiment * 1.0.1   2015-11-26 Bioconductor
##  XVector              * 0.10.0  2015-11-26 Bioconductor
##  yaml                   2.1.13  2014-06-12 CRAN (R 3.2.0)
##  zlibbioc               1.16.0  2015-11-26 Bioconductor
```

Normalize Library counts

Tomas Bjorklund

Wed Jan 13 13:15:41 2016

This workflow normalizes read counts between samples.

```
suppressPackageStartupMessages(library(knitr))
suppressPackageStartupMessages(library(GenomicAlignments))
suppressPackageStartupMessages(library(data.table))
suppressPackageStartupMessages(library(scales))
suppressPackageStartupMessages(library(ShortRead))
suppressPackageStartupMessages(library(multicore))
suppressPackageStartupMessages(library(plyr))
suppressPackageStartupMessages(library(Hmisc))
```

Generate load list and grouping names

```
in.names.all <- list.files("output", pattern="*.rds", full.names=TRUE)
load.list <- read.table("input/loadlist.txt", header = FALSE, skip = 0, sep="\t",
                        stringsAsFactors = FALSE, fill=TRUE)
colnames(load.list) <- c("Name", "BaseName","GroupName")
load.list <- rbind(load.list,c("completeLibraryRanges","","totalLib"))
load.list <- load.list[-grep("Untreat",load.list$Name),]

select.Cases <- c(unlist(sapply(load.list$Name, function(x) grep(x,in.names.all), simplify = TRUE)))

(in.names.all <- in.names.all[select.Cases])
```

```
##  [1] "output/found.RatNr18_4wks_1000x_Sc-12.rds"
##  [2] "output/found.RatNr2_4wks_100x_Ctx-1.rds"
##  [3] "output/found.RatNr2_4wks_100x_SN-2.rds"
##  [4] "output/found.RatNr2_4wks_100x_Str-3.rds"
##  [5] "output/found.RatNr2_4wks_100x_Th-4.rds"
##  [6] "output/found.RatNr5_4wks_100x_Mu-5.rds"
##  [7] "output/found.RatNr5_4wks_100x_Sc-6.rds"
##  [8] "output/found.RatNr13_4wks_1000x_Ctx-7.rds"
##  [9] "output/found.RatNr13_4wks_1000x_SN-8.rds"
## [10] "output/found.RatNr13_4wks_1000x_Str-9.rds"
## [11] "output/found.RatNr13_4wks_1000x_Th-10.rds"
## [12] "output/found.RatNr18_4wks_1000x_Mu-11.rds"
## [13] "output/found.RatNr7_100x_SN-1.rds"
## [14] "output/found.RatNr7_100x_Ctx-2.rds"
## [15] "output/found.RatNr7_100x_Str-3.rds"
## [16] "output/found.RatNr7_100x_Th-4.rds"
## [17] "output/found.RatNr1_100x_SN-5.rds"
## [18] "output/found.RatNr1_100x_Ctx-6.rds"
## [19] "output/found.RatNr1_100x_Str-7.rds"
## [20] "output/found.RatNr1_100x_Th-8.rds"
## [21] "output/found.RatNr8_100x_SN-9.rds"
## [22] "output/found.RatNr8_100x_Ctx-10.rds"
## [23] "output/found.RatNr8_100x_Str-11.rds"
```

```
## [24] "output/found.RatNr8_100x_Th-12.rds"
## [25] "output/found.RatNr15_1000x_SN-13.rds"
## [26] "output/found.RatNr15_1000x_Ctx-14.rds"
## [27] "output/found.RatNr15_1000x_Str-15.rds"
## [28] "output/found.RatNr15_1000x_Th-16.rds"
## [29] "output/found.RatNr21_1000x_SN-17.rds"
## [30] "output/found.RatNr21_1000x_Ctx-18.rds"
## [31] "output/found.RatNr21_1000x_Str-19.rds"
## [32] "output/found.RatNr21_1000x_Th-20.rds"
## [33] "output/found.RatNr19_1000x_Ctx-21.rds"
## [34] "output/found.RatNr19_1000x_Str-22.rds"
## [35] "output/found.RatNr19_1000x_Th-23.rds"
## [36] "output/found.RatNr20_1000x_Str-24.rds"
## [37] "output/found.RatNr20_1000x_Th-25.rds"
## [38] "output/found.Cells293Nr2_1000x_cDNA-26.rds"
## [39] "output/found.Cells293Nr3_100x_cDNA-27.rds"
## [40] "output/found.primNeuronsNr6_1000x_cDNA-28.rds"
## [41] "output/found.primNeuronsNr7_100x_cDNA-29.rds"
## [42] "output/found.primNeuronsNr6_1000x_RNA-30.rds"
## [43] "output/completeLibraryRanges.rds"
```

```
grouping <- data.frame(Sample=gsub("-","_",gsub("found.|(output/)|(.rds)", "", in.names.all)),
                       Group=load.list[match(names(select.Cases),load.list$Name),"GroupName"],
                       stringsAsFactors = FALSE)
```

Load the desired alignment files and annotating group

```
loadRDS <- function(in.name) {
  #in.name <- in.names.all[3]
  this.sample <- readRDS(in.name)
  this.name <- gsub("-","_",gsub("found.|(output/)|(.rds)", "", in.name))
  this.group <- grouping[match(this.name,grouping$Sample),"Group"]

  if (this.name == "completeLibraryRanges"){
    mcols(this.sample) <- cbind(mcols(this.sample),
                                data.frame(RNAcount=mcols(this.sample)$tCount,
                                           Sample = this.name, Group=this.group,
                                           stringsAsFactors = FALSE))
    #This is required, as the total library alignment does not have any RNA counts
  } else {
    mcols(this.sample) <- cbind(mcols(this.sample),
                                data.frame(Sample = this.name, Group=this.group,
                                           stringsAsFactors = FALSE))
  }
  return(this.sample)
}

out.range <- lapply(in.names.all, loadRDS)
```

Normalizing read counts to correct for variable read depth

```
readCounts <- lapply(out.range, function(x) sum(mcols(x)$RNAcount))
maxCount <- max(unlist(readCounts))
readCounts <- lapply(readCounts, function(x) maxCount/x)

makeNormCount <- function(inIndex){
  #inIndex <- 38
  thisRange <- out.range[[inIndex]]
  mcols(thisRange)$RNAcount <- mcols(thisRange)$RNAcount*readCounts[[inIndex]]
  return(thisRange)
  }


out.range <- lapply(1:length(readCounts), makeNormCount)
```

Flatten into one GAlignments object

```
out.range <- do.call(GAlignmentsList,unlist(out.range))
out.range <- cbind(unlist(out.range))[[1]]
mcols(out.range)$NormCount <- 1
out.range <- out.range[mcols(out.range)$Mode == "Def"] #Selects only defined i.e., trusted reads
```

Split the GAlignments into list based on group

```
out.range.split <- split(out.range,c(mcols(out.range)$Group))
out.range.split <- lapply(out.range.split, function(x) split(x,seqnames(x)))
out.range.split <- mclapply(out.range.split, function(x) lapply(x, function(y) split(y,names(y))),
                            mc.preschedule = TRUE, mc.cores = detectCores())

MergeCounts <- function(inRanges) {
outRanges <- inRanges[1]
mcols(outRanges) <- data.frame(structure=mcols(inRanges)$structure[1],
                               Group=mcols(inRanges)$Group[1],
                               bitScore=sum(mcols(inRanges)$bitScore*mcols(inRanges)$tCount)/sum(mcols(inRa
                               mismatches=median(mcols(inRanges)$mismatches),
                               mCount=sum(mcols(inRanges)$mCount),
                               tCount=sum(mcols(inRanges)$tCount),
                               BC=paste(unique(mcols(inRanges)$BC), collapse = ","),
                               Animals=paste(unique(mcols(inRanges)$Sample), collapse = ","),
                               RNAcount=sum(mcols(inRanges)$RNAcount),
                               NormCount=log2(sum(mcols(inRanges)$RNAcount)+1)*length(inRanges),
                               stringsAsFactors=FALSE)
return(outRanges)
}

out.range.split <- lapply(out.range.split, function(x) mclapply(x, function(y) lapply(y,MergeCounts), mc.pre

out.range.split <- mclapply(out.range.split,function(x) unlist(do.call(GAlignmentsList,unlist(x)), use.names
out.range.split <- unlist(do.call(GAlignmentsList,unlist(out.range.split)), use.names=FALSE)
saveRDS(out.range.split, file="data/normalizedSampleRangesDefined.RDS")
```

Pairwise sample analysis output

Tomas Bjorklund

Wed Jan 13 13:31:15 2016

This is the final script presenting top candidates and overview plots.

```
suppressPackageStartupMessages(library(knitr))
```

Generation of infective library

```
all.samples <- readRDS("data/normalizedSampleRangesDefined.RDS")
total.AAV.samples <- all.samples[!(mcols(all.samples)$Group %in% "totalLib")]
total.AAV.samples <- total.AAV.samples[-grep("4wks",mcols(total.AAV.samples)$Group)]
mcols(total.AAV.samples)$Group <- "infectiveLib"
all.samples <- append(all.samples,total.AAV.samples)
```

Plotting function

```
plotPair <- function(topSample,bottomSample,filterBC=FALSE,filterAnimal=FALSE,
                     AnimaladjustPlot=FALSE,NormalizePlot=TRUE) {
# Select samples
#====================

  topSample <- "CNS100x_Ctx"
  bottomSample <- "CNS100x_Str"
  filterBC <- FALSE
  filterAnimal <- FALSE
  AnimaladjustPlot <- FALSE
  NormalizePlot <- TRUE

fill.values <- eval(parse(text=paste("c(", topSample,"= rgb(38,64,135, maxColorValue = 255), ",
                                     bottomSample,"= rgb(157,190,217, maxColorValue = 255))",sep="")))

select.samples <- all.samples[mcols(all.samples)$Group %in% names(fill.values)]
trim.names <- data.table(GeneName=levels(seqnames(select.samples)))
trim.names[, c("Category", "Protein", "Origin",
               "Extra", "Number","GeneName") := tstrsplit(GeneName, ",", fixed=TRUE)]
trim.names$GeneName <- gsub("/|_","-",trim.names$GeneName)
levels(seqnames(select.samples)) <- trim.names$GeneName

geneTable <- data.frame(seqlengths(select.samples))
colnames(geneTable) <- "SeqLength"
geneTable$GeneName <- row.names(geneTable)

plot.data <- data.frame(GeneName=seqnames(select.samples),
                        Group=factor(mcols(select.samples)$Group, levels = names(fill.values)),
                        AApos=start(select.samples)+(width(select.samples)/2),
                        Animals=mcols(select.samples)$Animals,
                        BC=mcols(select.samples)$BC,
```

```
                        RNAcount=mcols(select.samples)$RNAcount,
                        NormCount=mcols(select.samples)$NormCount,
                        stringsAsFactors = FALSE)
plot.data <- merge(plot.data,geneTable)
plot.data$AAproc <- (plot.data$AApos/plot.data$SeqLength)*100

#====================
#Binning of data
#====================

size.bin <- 2
FullLength <- 100
position <- seq(0,FullLength,size.bin)
plot.data.dt <- data.table(plot.data)
plot.data.dt[,bin:=findInterval(AAproc, position)]
plot.data.bin <- plot.data.dt[, list(.N,SeqLength=min(SeqLength),
                                     AAproc = position[findInterval(min(AAproc),position)],
                                     BCmean=unlist(lapply(strsplit(paste(BC, collapse=","), ","),functio
                                     AnimalCount = length(table(strsplit(paste(t(Animals), collapse=",")
                                     NormCount = log2((sum(RNAcount)/SeqLength*FullLength)+1)
                                     ), by=c("bin","GeneName","Group")]
#====================
#Filtration parameters
#====================

if (NormalizePlot) {
  plot.data.bin[plot.data.bin$Group == names(fill.values)[1]]$NormCount <- plot.data.bin[plot.data.bin$Grou
  plot.data.bin[plot.data.bin$Group == names(fill.values)[2]]$NormCount <- plot.data.bin[plot.data.bin$Grou
}

if (filterBC) {
  plot.data.bin <- plot.data.bin[plot.data.bin$BCmean > 1,]
  select.samples <- select.samples[mcols(select.samples)$BC>1]
}

if (filterAnimal) {
  plot.data.bin <- plot.data.bin[plot.data.bin$AnimalCount > 1,]
}

if (AnimaladjustPlot) {
  plot.data.bin$NormCount <- plot.data.bin$NormCount*plot.data.bin$AnimalCount/plot.data.bin$SeqLength*Full
}

plot.data.bin[plot.data.bin$Group == names(fill.values)[2]]$NormCount <- plot.data.bin[plot.data.bin$Group =


#====================
# Sort and select top samples
#====================


select.samples.gr <- granges(select.samples)

mcols(select.samples.gr) <- cbind(mcols(select.samples)[,c(1,2,4,5,6,7,10)],
                                  DataFrame(Animals=unlist(lapply(strsplit(mcols(select.samples)$Animals, "

o = order(-mcols(select.samples.gr)$NormCount)
```

```
select.samples.gr <- select.samples.gr[o]
top.sample <- select.samples.gr[mcols(select.samples.gr)$Group %in% names(fill.values)[1]]
bottom.sample <- select.samples.gr[mcols(select.samples.gr)$Group %in% names(fill.values)[2]]

if (length(top.sample) >=1){
  out <- top.sample
  out.2 <- data.table(Fragment=names(out),
                      SeqCount=0,
                      Gene=as.character(seqnames(out)),
                      startAA=(start(out)+2)/3)
  out.2 <- data.frame(out.2,as.data.frame(mcols(out)))
  out.2$BC <- unlist(lapply(strsplit(out.2$BC, ","),function(x) length(unique(x))))
  out.2[match(names(table(out.2$Fragment)),out.2$Fragment),"SeqCount"] <- as.integer(table(out.2$Fragment))
  out.2 <- out.2[out.2$SeqCount>=1,]
  setnames(out.2, "Fragment", paste(topSample,"- Fragment"))
  top.sample.out.1 <- out.2[1:min(15,nrow(out.2)),c(1,3)]
  top.sample.out.2 <- out.2[1:min(15,nrow(out.2)),c(-1,-2,-6)]
} else {top.sample.out.1 <- top.sample.out.2 <- NA}

if (length(bottom.sample) >=1) {
  out <- bottom.sample
  out.2 <- data.table(Fragment=names(out),
                      SeqCount=0,
                      Gene=as.character(seqnames(out)),
                      startAA=(start(out)+2)/3)
  out.2 <- data.frame(out.2,as.data.frame(mcols(out)))
  out.2$BC <- unlist(lapply(strsplit(out.2$BC, ","),function(x) length(unique(x))))
  out.2[match(names(table(out.2$Fragment)),out.2$Fragment),"SeqCount"] <- as.integer(table(out.2$Fragment))
  out.2 <- out.2[out.2$SeqCount>=1,]
  setnames(out.2, "Fragment", paste(bottomSample,"- Fragment"))
  bottom.sample.out.1 <- out.2[1:min(15,nrow(out.2)),c(1,3,6)]
  bottom.sample.out.2 <-out.2[1:min(15,nrow(out.2)),c(-1,-2,-6)]
} else {bottom.sample.out.1 <- bottom.sample.out.2 <- NA}

#===================
#Output plot
#===================

plot.out <- ggplot(plot.data.bin,aes(x=AAproc,y=NormCount, fill = Group)) +
  geom_bar(stat="identity", position="identity")+theme_bw() +
  scale_fill_manual(name = "Library", values = fill.values) +
  scale_colour_manual(name = "Library", values = fill.values) +
  scale_x_continuous(limit=c(0,100), breaks=c(seq(0,100,20)), expand =c(0,0)) +
  facet_wrap(~ GeneName, ncol=5)+
  theme(plot.margin=unit(x=c(0,0,0,0),units="mm"),
        legend.position="bottom",
        legend.margin=unit(-0.5,"cm"),
        legend.key.height=unit(0, "cm"),
        plot.background=element_rect(fill="white"),
        axis.text = element_text(size = rel(0.5)),
        axis.ticks = element_line(size = rel(0.5)),
        axis.ticks.length = unit(.05, "cm"),
        strip.text.x = element_text(size = rel(0.5), colour = "black",
                                    angle = 0, lineheight=0.1, vjust=0.1),
        strip.background = element_blank(),
        strip.background = element_rect(size = 0),
        panel.margin.y = unit(-0.15, "cm"),
```

```
        panel.margin.x = unit(0, "cm"))

out.list <- list(plot=plot.out,
                 plotBin=plot.data.bin,
                 top1=top.sample.out.1,
                 top2=top.sample.out.2,
                 bottom1=bottom.sample.out.1,
                 bottom2=bottom.sample.out.2)

return(out.list)
}
```

Analyze samples

```
out.plot.list <- plotPair("totalLib","infectiveLib",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

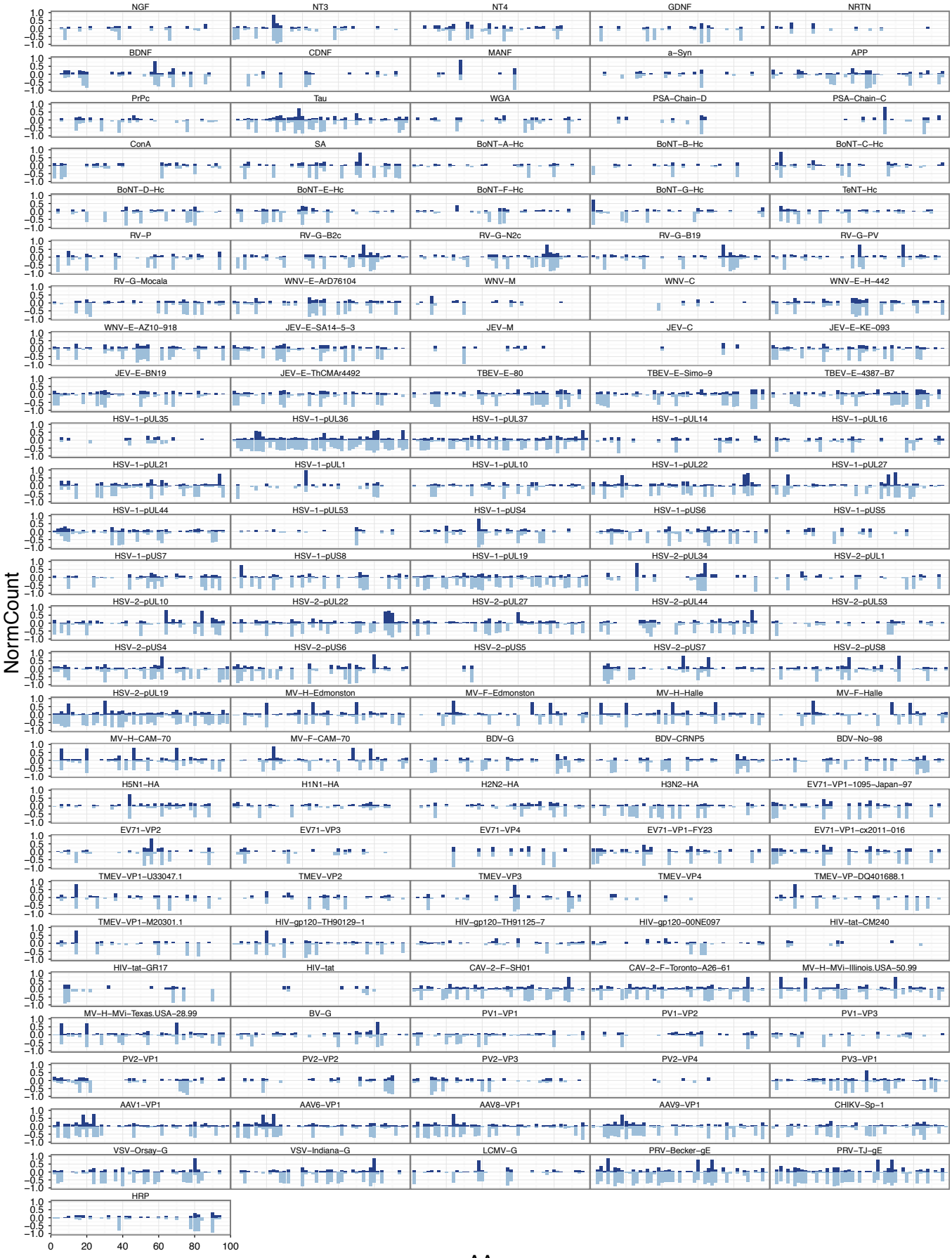
```
knitr::kable(out.plot.list$bottom2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-1-pUL36	1519	22aa	0.0	26	25	6	94.41806	3
2	PV1-VP2	236	14aa	0.0	13	17	5	83.54495	3
3	HSV-2-pUL27	393	14aa	1.0	16	21	5	80.69311	3
4	Tau	241	14aaG4S	0.5	16	25	4	68.21062	3
5	HSV-1-pUL21	75	14aa	0.5	20	35	4	64.98599	3
6	HSV-2-pUS6	275	14aa	1.0	31	44	4	64.03911	3
7	HSV-2-pUL19	419	14aa	0.0	19	29	4	63.95275	3
8	HRP	250	14aaG4S	0.0	15	22	4	59.97958	3
9	HSV-1-pUL36	2552	14aa	0.5	33	45	4	59.55352	2
10	HSV-2-pUS8	235	14aaA5	0.0	15	18	4	59.51165	3
11	VSV-Orsay-G	387	14aaA5	0.0	24	32	4	55.92369	3
13	TMEV-VP2	133	22aa	0.0	15	20	4	54.28445	2
14	HSV-1-pUL37	920	14aa	0.0	8	15	3	52.57127	2
15	HSV-1-pUL36	2521	22aa	0.0	13	17	3	51.46529	3
16	VSV-Orsay-G	192	14aa	0.0	11	18	3	51.34442	2

```
out.plot.list <- plotPair("CNS100x_Th","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
out.plot.list <- plotPair("CNS100x_Th","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=TRUE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```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NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$plotBin[1:10,], format = "markdown")
```

bin	GeneName	Group	N	SeqLength	AAproc	BCmean	AnimalCount	NormCount
32	AAV1-VP1	CNS100x_Str	3	2208	62	4	2	-0.5991837
32	AAV1-VP1	CNS100x_Str	3	2208	62	4	2	-0.5991837
32	AAV1-VP1	CNS100x_Str	3	2208	62	4	2	-0.5991837
6	AAV1-VP1	CNS100x_Str	5	2208	10	7	3	-0.7834970
6	AAV1-VP1	CNS100x_Str	5	2208	10	7	3	-0.7834970
6	AAV1-VP1	CNS100x_Str	5	2208	10	7	3	-0.7834970
6	AAV1-VP1	CNS100x_Str	5	2208	10	7	3	-0.7834970
6	AAV1-VP1	CNS100x_Str	5	2208	10	7	3	-0.7834970
36	AAV1-VP1	CNS100x_Str	2	2208	70	2	2	-0.1655803
36	AAV1-VP1	CNS100x_Str	2	2208	70	2	2	-0.1655803

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
knitr::kable(out.plot.list$bottom2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-1-pUL36	1519	22aa	0.0	26	25	6	94.41806	3
2	PV1-VP2	236	14aa	0.0	13	17	5	83.54495	3
3	HSV-2-pUL27	393	14aa	1.0	16	21	5	80.69311	3

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
4	Tau	241	14aaG4S	0.5	16	25	4	68.21062	3
5	HSV-1-pUL21	75	14aa	0.5	20	35	4	64.98599	3
6	HSV-2-pUS6	275	14aa	1.0	31	44	4	64.03911	3
7	HSV-2-pUL19	419	14aa	0.0	19	29	4	63.95275	3
8	HRP	250	14aaG4S	0.0	15	22	4	59.97958	3
9	HSV-1-pUL36	2552	14aa	0.5	33	45	4	59.55352	2
10	HSV-2-pUS8	235	14aaA5	0.0	15	18	4	59.51165	3
11	VSV-Orsay-G	387	14aaA5	0.0	24	32	4	55.92369	3
13	TMEV-VP2	133	22aa	0.0	15	20	4	54.28445	2
14	HSV-1-pUL37	920	14aa	0.0	8	15	3	52.57127	2
15	HSV-1-pUL36	2521	22aa	0.0	13	17	3	51.46529	3
16	VSV-Orsay-G	192	14aa	0.0	11	18	3	51.34442	2

```
out.plot.list <- plotPair("CNS100x_Ctx","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
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HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
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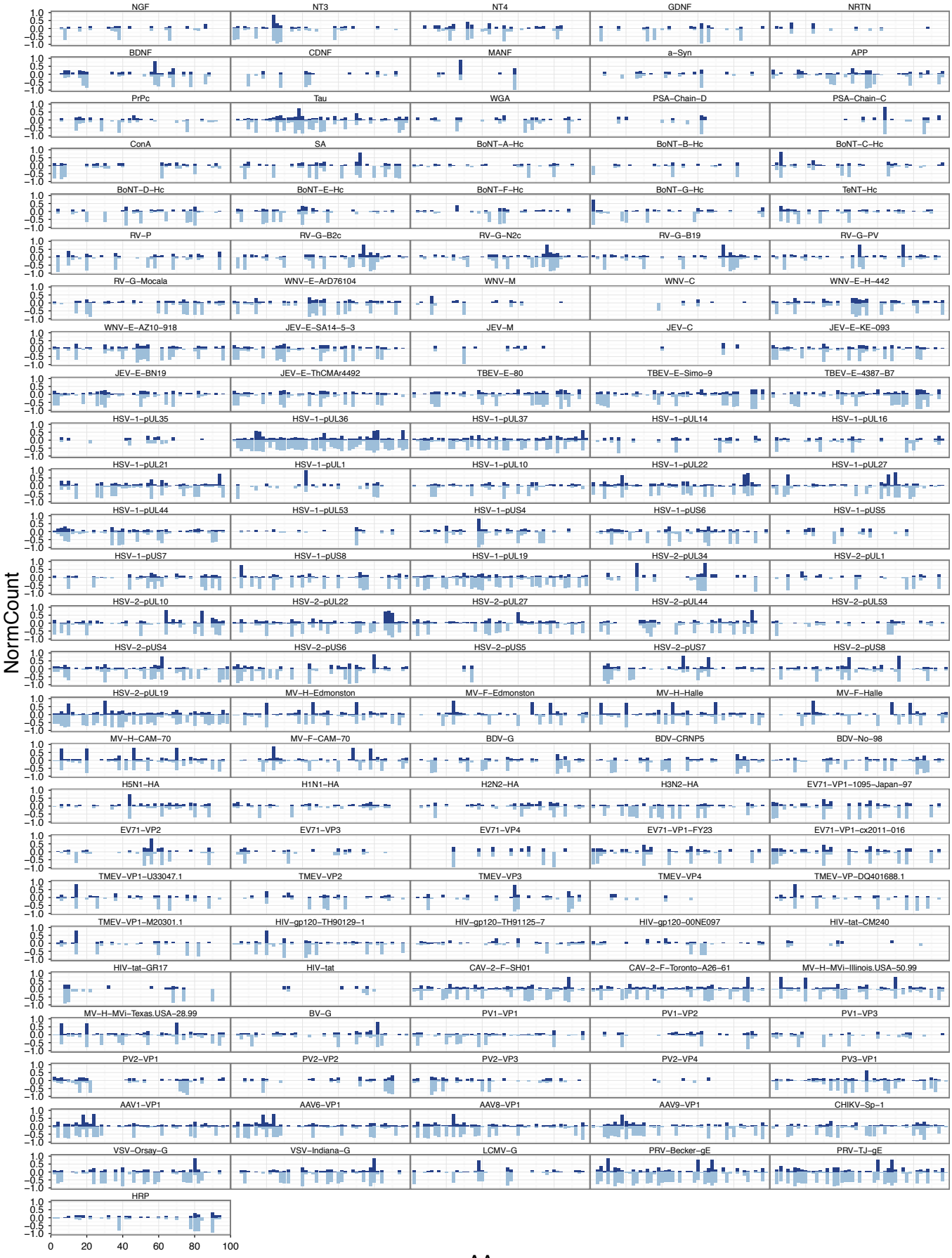
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
out.plot.list <- plotPair("CNS100x_Ctx","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=TRUE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```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NRTN
BDNF
CDNF
MANF
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APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
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HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
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HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
out.plot.list <- plotPair("CNS100x_SN","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
out.plot.list <- plotPair("CNS100x_SN","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=TRUE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
out.plot.list <- plotPair("CNS1000x_Th","CNS1000x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
knitr::kable(out.plot.list$bottom2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-1-pUL36	1519	22aa	0.0	26	25	6	94.41806	3
2	PV1-VP2	236	14aa	0.0	13	17	5	83.54495	3
3	HSV-2-pUL27	393	14aa	1.0	16	21	5	80.69311	3
4	Tau	241	14aaG4S	0.5	16	25	4	68.21062	3
5	HSV-1-pUL21	75	14aa	0.5	20	35	4	64.98599	3
6	HSV-2-pUS6	275	14aa	1.0	31	44	4	64.03911	3
7	HSV-2-pUL19	419	14aa	0.0	19	29	4	63.95275	3
8	HRP	250	14aaG4S	0.0	15	22	4	59.97958	3
9	HSV-1-pUL36	2552	14aa	0.5	33	45	4	59.55352	2
10	HSV-2-pUS8	235	14aaA5	0.0	15	18	4	59.51165	3
11	VSV-Orsay-G	387	14aaA5	0.0	24	32	4	55.92369	3
13	TMEV-VP2	133	22aa	0.0	15	20	4	54.28445	2
14	HSV-1-pUL37	920	14aa	0.0	8	15	3	52.57127	2
15	HSV-1-pUL36	2521	22aa	0.0	13	17	3	51.46529	3
16	VSV-Orsay-G	192	14aa	0.0	11	18	3	51.34442	2

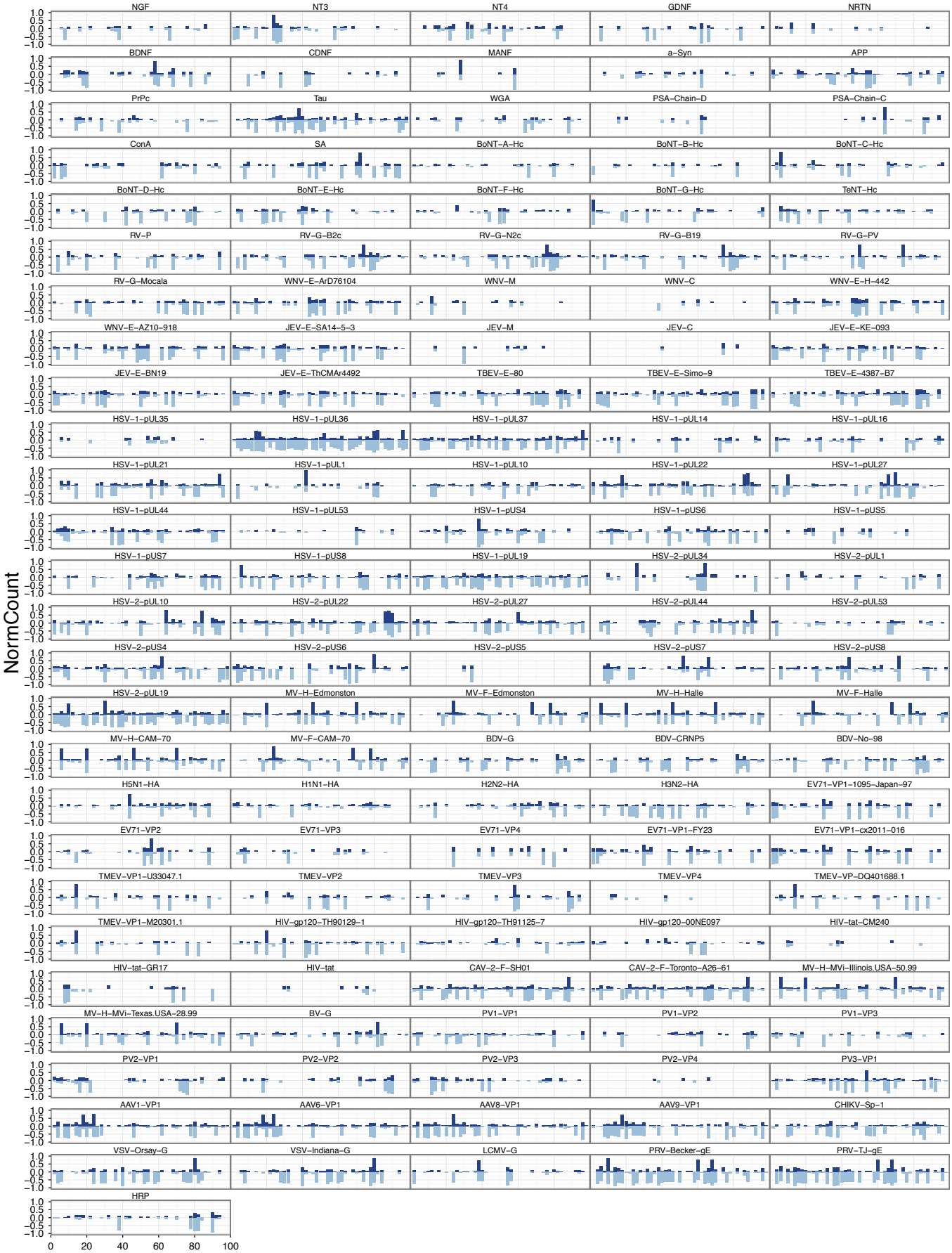
```
out.plot.list <- plotPair("CNS1000x_Ctx","CNS1000x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
out.plot.list <- plotPair("CNS1000x_SN","CNS1000x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
out.plot.list <- plotPair("CNS1000x_Str","CNS100x_Str",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)

out.plot.list <- plotPair("CNS1000x_Th","CNS100x_Th",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)

out.plot.list <- plotPair("CNS1000x_Ctx","CNS100x_Ctx",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=TRUE,
                          NormalizePlot=TRUE)

out.plot.list <- plotPair("CNS1000x_SN","CNS100x_SN",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)

out.plot.list <- plotPair("PrimN_1000x","PrimN_100x",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
```

```
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

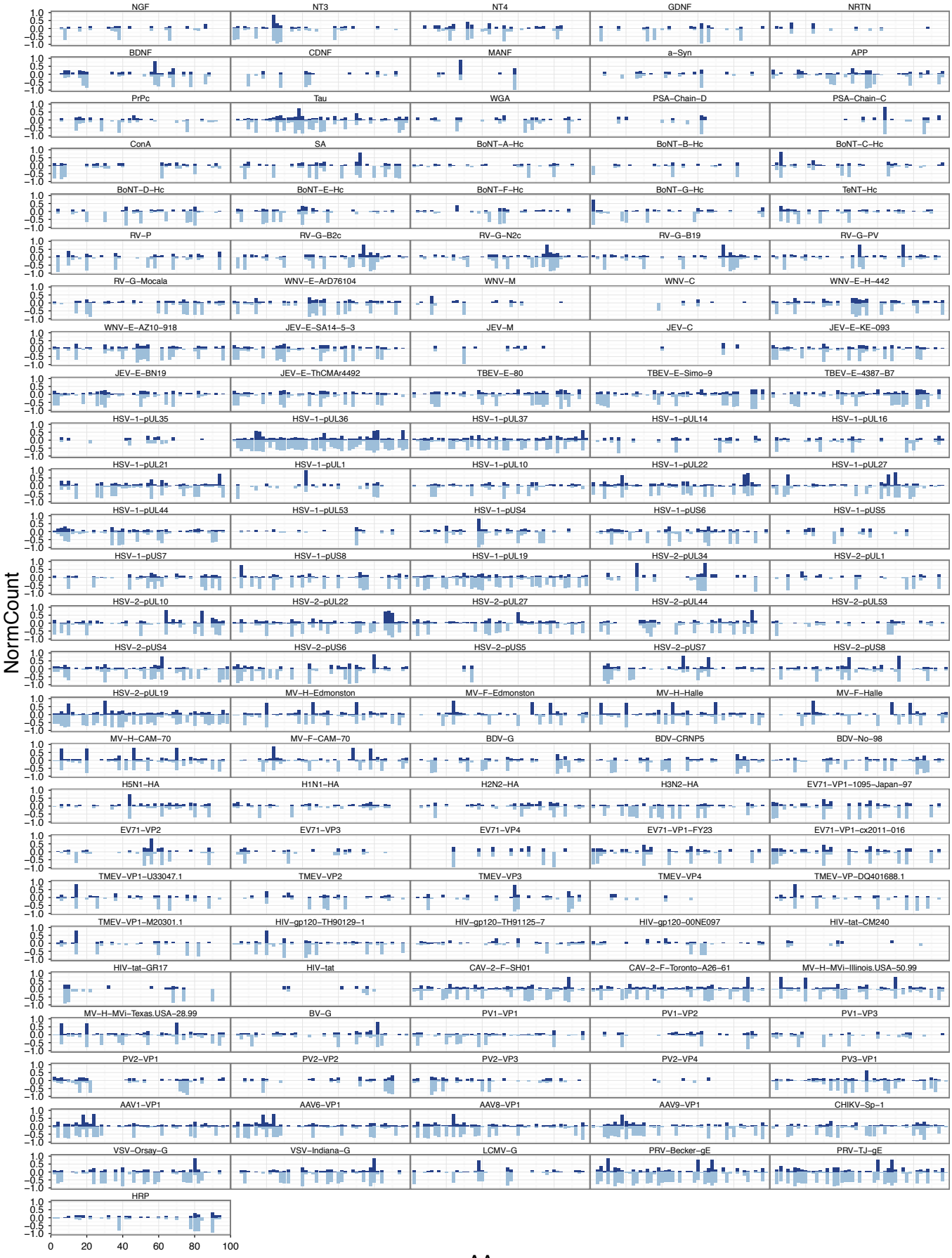
```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
knitr::kable(out.plot.list$bottom2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-1-pUL36	1519	22aa	0.0	26	25	6	94.41806	3
2	PV1-VP2	236	14aa	0.0	13	17	5	83.54495	3
3	HSV-2-pUL27	393	14aa	1.0	16	21	5	80.69311	3
4	Tau	241	14aaG4S	0.5	16	25	4	68.21062	3
5	HSV-1-pUL21	75	14aa	0.5	20	35	4	64.98599	3
6	HSV-2-pUS6	275	14aa	1.0	31	44	4	64.03911	3
7	HSV-2-pUL19	419	14aa	0.0	19	29	4	63.95275	3
8	HRP	250	14aaG4S	0.0	15	22	4	59.97958	3
9	HSV-1-pUL36	2552	14aa	0.5	33	45	4	59.55352	2
10	HSV-2-pUS8	235	14aaA5	0.0	15	18	4	59.51165	3
11	VSV-Orsay-G	387	14aaA5	0.0	24	32	4	55.92369	3
13	TMEV-VP2	133	22aa	0.0	15	20	4	54.28445	2
14	HSV-1-pUL37	920	14aa	0.0	8	15	3	52.57127	2
15	HSV-1-pUL36	2521	22aa	0.0	13	17	3	51.46529	3
16	VSV-Orsay-G	192	14aa	0.0	11	18	3	51.34442	2

```
mcols(all.samples)$Group[mcols(all.samples)$Group== "293T_1000x"] <- "H293T_1000x"
mcols(all.samples)$Group[mcols(all.samples)$Group== "293T_100x"] <- "H293T_100x"

out.plot.list <- plotPair("H293T_1000x","H293T_100x",
                          filterBC=FALSE,
                          filterAnimal=FALSE,
                          AnimaladjustPlot=FALSE,
                          NormalizePlot=TRUE)
out.plot.list$plot
```


NGF
NT3
NT4
GDNF
NRTN
BDNF
CDNF
MANF
a−Syn
APP
PrPc
Tau
WGA
PSA−Chain−D
PSA−Chain−C
ConA
SA
BoNT−A−Hc
BoNT−B−Hc
BoNT−C−Hc
BoNT−D−Hc
BoNT−E−Hc
BoNT−F−Hc
BoNT−G−Hc
TeNT−Hc
RV−P
RV−G−B2c
RV−G−N2c
RV−G−B19
RV−G−PV
RV−G−Mocala
WNV−E−ArD76104
WNV−M
WNV−C
WNV−E−H−442
WNV−E−AZ10−918
JEV−E−SA14−5−3
JEV−M
JEV−C
JEV−E−KE−093
JEV−E−BN19
JEV−E−ThCMAr4492
TBEV−E−80
TBEV−E−Simo−9
TBEV−E−4387−B7
HSV−1−pUL35
HSV−1−pUL36
HSV−1−pUL37
HSV−1−pUL14
HSV−1−pUL16
HSV−1−pUL21
HSV−1−pUL1
HSV−1−pUL10
HSV−1−pUL22
HSV−1−pUL27
HSV−1−pUL44
HSV−1−pUL53
HSV−1−pUS4
HSV−1−pUS6
HSV−1−pUS5
HSV−1−pUS7
HSV−1−pUS8
HSV−1−pUL19
HSV−2−pUL34
HSV−2−pUL1
HSV−2−pUL10
HSV−2−pUL22
HSV−2−pUL27
HSV−2−pUL44
HSV−2−pUL53
HSV−2−pUS4
HSV−2−pUS6
HSV−2−pUS5
HSV−2−pUS7
HSV−2−pUS8
HSV−2−pUL19
MV−H−Edmonston
MV−F−Edmonston
MV−H−Halle
MV−F−Halle
MV−H−CAM−70
MV−F−CAM−70
BDV−G
BDV−CRNP5
BDV−No−98
H5N1−HA
H1N1−HA
H2N2−HA
H3N2−HA
EV71−VP1−1095−Japan−97
EV71−VP2
EV71−VP3
EV71−VP4
EV71−VP1−FY23
EV71−VP1−cx2011−016
TMEV−VP1−U33047.1
TMEV−VP2
TMEV−VP3
TMEV−VP4
TMEV−VP−DQ401688.1
TMEV−VP1−M20301.1
HIV−gp120−TH90129−1
HIV−gp120−TH91125−7
HIV−gp120−00NE097
HIV−tat−CM240
HIV−tat−GR17
HIV−tat
CAV−2−F−SH01
CAV−2−F−Toronto−A26−61
MV−H−MVi−Illinois.USA−50.99
MV−H−MVi−Texas.USA−28.99
BV−G
PV1−VP1
PV1−VP2
PV1−VP3
PV2−VP1
PV2−VP2
PV2−VP3
PV2−VP4
PV3−VP1
AAV1−VP1
AAV6−VP1
AAV8−VP1
AAV9−VP1
CHIKV−Sp−1
VSV−Orsay−G
VSV−Indiana−G
LCMV−G
PRV−Becker−gE
PRV−TJ−gE
HRP
1.0
0.5
0.0
−0.5
−1.0
0
20
40
60
80
100
NormCount
AAproc
Library
CNS100x_Ctx
CNS100x_Str

```
knitr::kable(out.plot.list$top2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-2-pUL22	743	14aa	0.0	15	36	5	95.79975	3
2	BV-G	318	14aa	0.0	25	36	5	89.79143	2
3	HSV-2-pUS7	194	14aa	1.5	31	47	4	72.82153	3
4	HSV-1-pUL22	160	14aaA5	0.5	19	30	4	68.82554	3
5	HSV-2-pUL19	419	14aa	0.0	7	7	3	63.54107	2
6	HSV-1-pUL1	88	14aaA5	0.0	14	17	3	63.13008	3
7	HSV-1-pUL27	628	14aaA5	1.0	15	22	3	60.86283	3
8	Tau	480	14aa	2.0	26	38	5	58.50914	2
9	HSV-2-pUS8	391	14aa	0.0	12	11	3	57.97992	3
10	BoNT-C-Hc	21	14aa	2.0	28	45	3	57.95954	3
11	MV-F-Edmonston	378	14aa	0.0	26	21	3	55.34030	3
14	HSV-1-pUL36	2601	14aa	0.0	10	14	3	54.69235	2
15	HSV-1-pUL21	499	14aa	0.0	8	9	3	54.41724	3
16	HSV-1-pUL27	608	14aa	0.0	16	22	3	54.32717	3
17	SA	177	14aa	0.0	11	13	3	54.07652	3

```
knitr::kable(out.plot.list$bottom2, format = "markdown")
```

	Gene	startAA	structure	mismatches	mCount	tCount	BC	NormCount	Animals
1	HSV-1-pUL36	1519	22aa	0.0	26	25	6	94.41806	3
2	PV1-VP2	236	14aa	0.0	13	17	5	83.54495	3
3	HSV-2-pUL27	393	14aa	1.0	16	21	5	80.69311	3
4	Tau	241	14aaG4S	0.5	16	25	4	68.21062	3
5	HSV-1-pUL21	75	14aa	0.5	20	35	4	64.98599	3
6	HSV-2-pUS6	275	14aa	1.0	31	44	4	64.03911	3
7	HSV-2-pUL19	419	14aa	0.0	19	29	4	63.95275	3
8	HRP	250	14aaG4S	0.0	15	22	4	59.97958	3
9	HSV-1-pUL36	2552	14aa	0.5	33	45	4	59.55352	2
10	HSV-2-pUS8	235	14aaA5	0.0	15	18	4	59.51165	3
11	VSV-Orsay-G	387	14aaA5	0.0	24	32	4	55.92369	3
13	TMEV-VP2	133	22aa	0.0	15	20	4	54.28445	2
14	HSV-1-pUL37	920	14aa	0.0	8	15	3	52.57127	2
15	HSV-1-pUL36	2521	22aa	0.0	13	17	3	51.46529	3
16	VSV-Orsay-G	192	14aa	0.0	11	18	3	51.34442	2

```
devtools::session_info()
```

```
Session info -------------------------------------------------------------

 setting  value
 version  R version 3.2.3 (2015-12-10)
 system   x86_64, linux-gnu
 ui       X11
 language en_US:en
 collate  en_US.UTF-8
 tz       <NA>
 date     2016-01-13

Packages -----------------------------------------------------------------

 package              * version  date       source
 acepack                1.3-3.3  2014-11-24 CRAN (R 3.2.0)
 AnnotationDbi          1.32.0   2015-11-26 Bioconductor
 Biobase              * 2.30.0   2015-11-26 Bioconductor
 BiocGenerics         * 0.16.1   2015-11-26 Bioconductor
 BiocInstaller          1.20.1   2015-11-26 Bioconductor
 BiocParallel           1.4.0    2015-11-26 Bioconductor
 biomaRt                2.26.1   2015-11-26 Bioconductor
 Biostrings           * 2.38.2   2015-11-26 Bioconductor
 biovizBase             1.18.0   2015-11-26 Bioconductor
 bitops                 1.0-6    2013-08-17 CRAN (R 3.2.0)
 BSgenome               1.38.0   2015-11-26 Bioconductor
 chron                  2.3-47   2015-06-24 CRAN (R 3.2.2)
 cluster                2.0.3    2015-07-21 CRAN (R 3.2.2)
 codetools              0.2-14   2015-07-15 CRAN (R 3.2.2)
 colorspace             1.2-6    2015-03-11 CRAN (R 3.2.0)
 data.table           * 1.9.6    2015-09-19 CRAN (R 3.2.2)
 DBI                    0.3.1    2014-09-24 CRAN (R 3.2.0)
 devtools             * 1.9.1    2015-09-11 CRAN (R 3.2.2)
 dichromat              2.0-0    2013-01-24 CRAN (R 3.2.0)
 digest                 0.6.8    2014-12-31 CRAN (R 3.2.0)
 doParallel           * 1.0.10   2015-10-14 CRAN (R 3.2.2)
 evaluate               0.8      2015-09-18 CRAN (R 3.2.2)
 foreach              * 1.4.3    2015-10-13 CRAN (R 3.2.2)
 foreign                0.8-66   2015-08-19 CRAN (R 3.2.2)
 formatR                1.2.1    2015-09-18 CRAN (R 3.2.2)
 Formula                1.2-1    2015-04-07 CRAN (R 3.2.0)
 futile.logger          1.4.1    2015-04-20 CRAN (R 3.2.0)
 futile.options         1.0.0    2010-04-06 CRAN (R 3.2.0)
 GenomeInfoDb         * 1.6.1    2015-11-26 Bioconductor
 GenomicAlignments    * 1.6.1    2015-11-26 Bioconductor
 GenomicFeatures        1.22.5   2015-11-26 Bioconductor
 GenomicRanges        * 1.22.1   2015-11-26 Bioconductor
 GGally                 0.5.0    2014-12-02 CRAN (R 3.2.0)
 ggbio                * 1.18.1   2015-11-26 Bioconductor
 ggplot2              * 1.0.1    2015-03-17 CRAN (R 3.2.0)
 graph                  1.48.0   2015-11-26 Bioconductor
 gridExtra              2.0.0    2015-07-14 CRAN (R 3.2.2)
 gtable                 0.1.2    2012-12-05 CRAN (R 3.2.0)
 highr                  0.5.1    2015-09-18 CRAN (R 3.2.2)
 Hmisc                  3.17-0   2015-09-21 CRAN (R 3.2.2)
```

```
htmltools              0.2.6    2014-09-08 CRAN (R 3.2.0)
IRanges              * 2.4.4    2015-11-26 Bioconductor
iterators            * 1.0.8    2015-10-13 CRAN (R 3.2.2)
knitr                * 1.11     2015-08-14 CRAN (R 3.2.2)
labeling               0.3      2014-08-23 CRAN (R 3.2.0)
lambda.r               1.1.7    2015-03-20 CRAN (R 3.2.0)
lattice                0.20-33  2015-07-14 CRAN (R 3.2.2)
latticeExtra           0.6-26   2013-08-15 CRAN (R 3.2.0)
magrittr               1.5      2014-11-22 CRAN (R 3.2.2)
MASS                   7.3-45   2015-11-10 CRAN (R 3.2.2)
memoise                0.2.1    2014-04-22 CRAN (R 3.2.2)
munsell                0.4.2    2013-07-11 CRAN (R 3.2.0)
nnet                   7.3-11   2015-08-30 CRAN (R 3.2.2)
OrganismDbi            1.12.0   2015-11-26 Bioconductor
plyr                 * 1.8.3    2015-06-12 CRAN (R 3.2.2)
proto                  0.3-10   2012-12-22 CRAN (R 3.2.0)
RBGL                   1.46.0   2015-11-26 Bioconductor
RColorBrewer           1.1-2    2014-12-07 CRAN (R 3.2.0)
Rcpp                   0.12.2   2015-11-15 CRAN (R 3.2.2)
RCurl                  1.95-4.7 2015-06-30 CRAN (R 3.2.2)
reshape                0.8.5    2014-04-23 CRAN (R 3.2.0)
reshape2               1.4.1    2014-12-06 CRAN (R 3.2.0)
rmarkdown              0.8.1    2015-10-10 CRAN (R 3.2.2)
rpart                  4.1-10   2015-06-29 CRAN (R 3.2.2)
Rsamtools            * 1.22.0   2015-11-26 Bioconductor
RSQLite                1.0.0    2014-10-25 CRAN (R 3.2.0)
rtracklayer            1.30.1   2015-11-26 Bioconductor
S4Vectors            * 0.8.3    2015-11-26 Bioconductor
scales                 0.3.0    2015-08-25 CRAN (R 3.2.2)
stringi                1.0-1    2015-10-22 CRAN (R 3.2.2)
stringr                1.0.0    2015-04-30 CRAN (R 3.2.2)
SummarizedExperiment * 1.0.1    2015-11-26 Bioconductor
survival               2.38-3   2015-07-02 CRAN (R 3.2.2)
VariantAnnotation      1.16.3   2015-11-26 Bioconductor
XML                    3.98-1.3 2015-06-30 CRAN (R 3.2.2)
XVector              * 0.10.0   2015-11-26 Bioconductor
yaml                   2.1.13   2014-06-12 CRAN (R 3.2.0)
zlibbioc               1.16.0   2015-11-26 Bioconductor
```

Top 10 heatmap analysis output

Tomas Bjorklund

Wed Jan 13 13:34:59 2016

This is the final script presenting top 10 candidates as heatmap plots.

```
suppressPackageStartupMessages(library(knitr))
```

Setup parameters

```
select.samples <- readRDS("data/normalizedSampleRangesDefined.RDS")
mcols(select.samples)$Sequence <- names(select.samples)
names(select.samples) <- make.names(names(select.samples), unique=TRUE)
length.Table <- data.table(seqnames=names(seqlengths(select.samples)),
                           seqlength=seqlengths(select.samples), key="seqnames")
select.samples <- data.table(as.data.frame(select.samples), key="seqnames")
select.samples[,c("strand","qwidth","cigar","njunc","end"):=NULL]
select.samples <- select.samples[length.Table] #A data.table berge to match seglengths to their respective
```

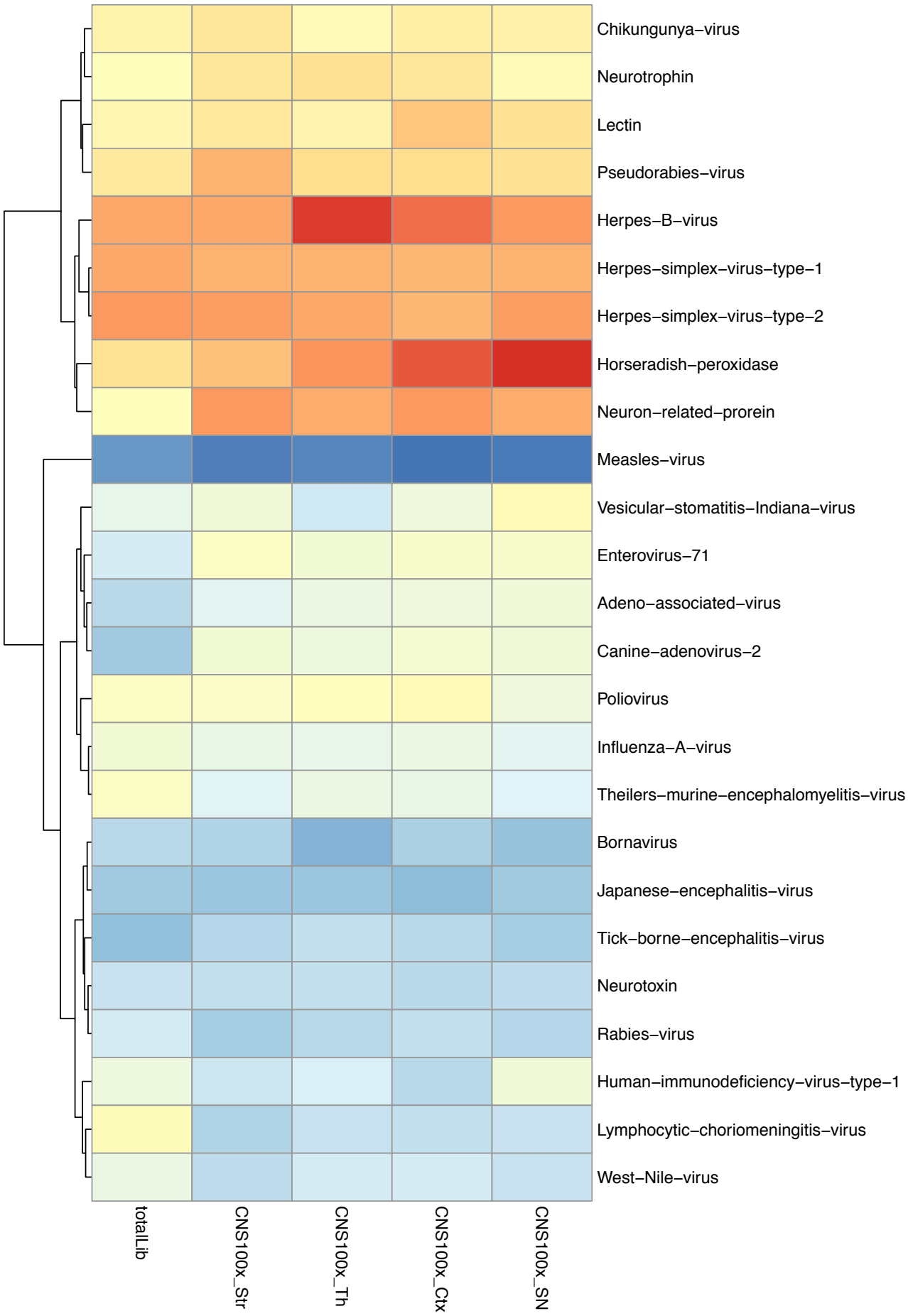
Selection of relevant samples

```
select.samples <- select.samples[-grep("4wks|PrimN_1000x_RNA",select.samples$Group),]
select.samples[, c("Category", "Protein", "Origin", "Extra", "Number","GeneName") := tstrsplit(seqnames, ","
select.samples[,c("seqnames","Extra"):=NULL]

select.samples.binCat <<- select.samples
setkeyv(select.samples.binCat,c("Group","Category"))
select.samples.binCat[,c("BCcount","NormCount"):=list(unlist(lapply(strsplit(paste(BC, collapse=","), ","),f
                                                      mean(NormCount)), by=key(select.samples.binCat)]
select.samples.binCat <- unique(select.samples.binCat)
select.samples.binCat <- select.samples.binCat[,c("Group","Category",
                                                  "BCcount","NormCount"), with = FALSE]
select.samples.binCat[,totBC:=sum(BCcount), by="Group"]
max.count <- max(select.samples.binCat$totBC)
select.samples.binCat[,BCcountN:=BCcount/totBC*max.count]

length.Table[, c("Category", "Protein", "Origin", "Extra", "Number","GeneName") := tstrsplit(seqnames, ",",
setkey(length.Table,"Category")
length.Table[,seqlength:=sum(seqlength), by="Category"]
length.Table <- length.Table[,c("Category","seqlength"), with = FALSE]
length.Table <- unique(length.Table)
setkey(select.samples.binCat,"Category")
select.samples.binCat <- select.samples.binCat[length.Table,nomatch=0]
select.samples.binCat[,Category:=gsub("/|_|'","-",Category)]
select.samples.binCat[,BCcountNseq:=BCcountN/seqlength]
select.samples.binCat[,NormCountBC:=BCcountNseq*NormCount]


select.samples.binGene <<- select.samples
```

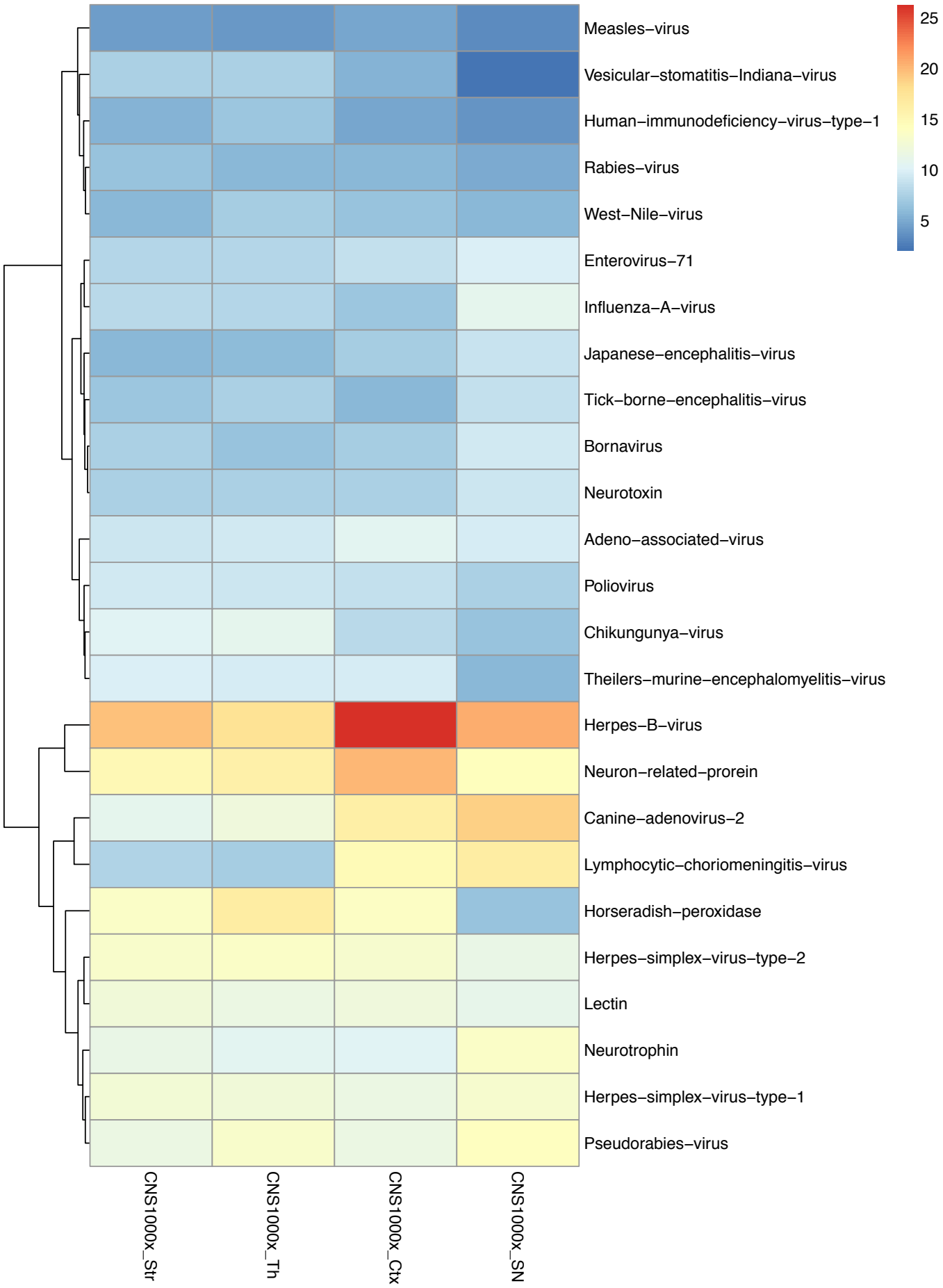
```
setkeyv(select.samples.binGene,c("Group","Category","GeneName"))
select.samples.binGene[,c("BCcount","NormCount"):=list(unlist(lapply(strsplit(paste(BC, collapse=","), ","),
                                                      mean(NormCount)), by=key(select.samples.binGene)]
select.samples.binGene <- unique(select.samples.binGene)
select.samples.binGene <- select.samples.binGene[,c("Group","GeneName","Category","BCcount","seqlength","Nor
select.samples.binGene[,GeneName:=gsub("/|_|'","-",GeneName)]
select.samples.binGene[,totBC:=sum(BCcount), by="Group"]
max.count <- max(select.samples.binGene$totBC)
select.samples.binGene[,BCcountN:=BCcount/totBC*max.count]
select.samples.binGene[,BCcountNseq:=BCcountN/seqlength]
select.samples.binGene[,NormCountBC:=BCcountNseq*NormCount]

select.samples.binPos <<- select.samples
setkeyv(select.samples.binPos,c("Group","structure","Sequence"))
select.samples.binPos <- unique(select.samples.binPos)
#Due to key, this removes replicates if identical sequence mapped to multiple genes

select.samples.binPos[,AA:=((start+2)/3)+floor((width/3)/2)]
setkeyv(select.samples.binPos,c("Group","Category","GeneName","AA"))
select.samples.binPos[,c("BCcount","NormCount","AnimalCount","mainStruct","mismatches"):=
                        list(unlist(lapply(strsplit(paste(BC, collapse=","), ","),function(x) length(unique(
                             mean(NormCount),
                             unlist(lapply(strsplit(paste(Animals, collapse=","), ","),function(x) length(un
                             paste(unique(structure), collapse=","),
                             median(mismatches)), by=key(select.samples.binPos)]

select.samples.binPos <- unique(select.samples.binPos)
select.samples.binPos <- select.samples.binPos[,c("Group","GeneName","AA","NormCount",
                                                  "BCcount","AnimalCount","mainStruct",
                                                  "mismatches","seqlength"), with = FALSE]
select.samples.binPos[,totBC:=sum(BCcount), by="Group"]
max.count <- max(select.samples.binPos$totBC)
select.samples.binPos[,BCcountN:=BCcount/totBC*max.count]
select.samples.binPos[,BCcountNanim:=BCcountN*AnimalCount]
select.samples.binPos[,BCcountNseq:=BCcountN/seqlength]
select.samples.binPos[,NormCountBC:=BCcountNanim*NormCount]
select.samples.binPos[,GeneAA:=paste(GeneName," [",AA,"]",sep="")]
```

Plot Heatmaps split by Category

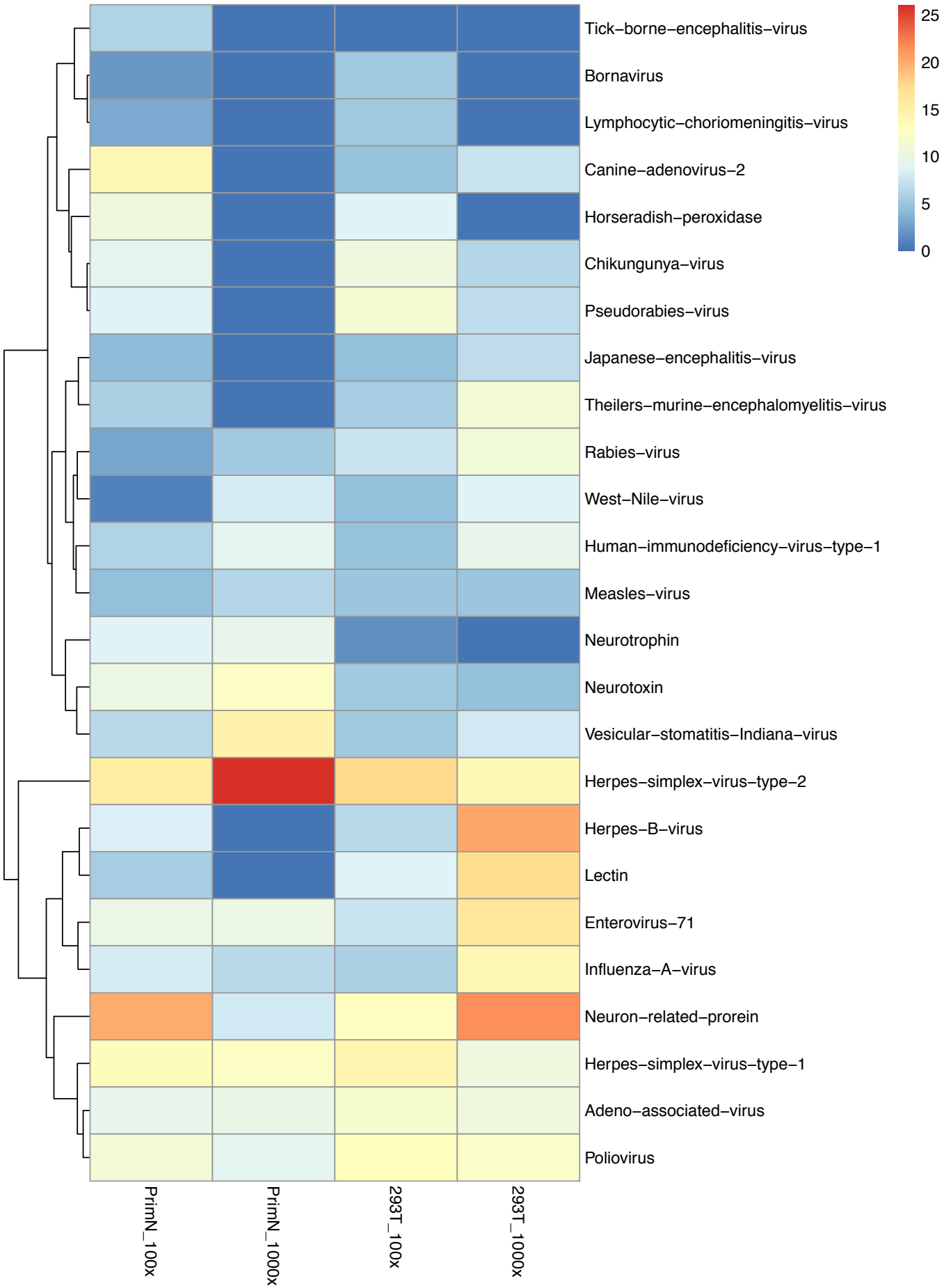
```
plotCategory <- function(select.samples.table,plot.col,sample.select){
setkey(select.samples.table,Group)
select.samples.select <- select.samples.table[sample.select]
eval(parse(text=paste("setorder(select.samples.select,Group, -", plot.col,")", sep="")))
select.samples.matrix <- acast(select.samples.select, Category~Group, value.var=plot.col)
#Utilizes reshape 2 to make matrix for heatmap

select.samples.matrix[is.na(select.samples.matrix)] <- 0
select.samples.matrix <- select.samples.matrix[,sample.select]
return(pheatmap(select.samples.matrix, cluster_rows=TRUE, show_rownames=TRUE, cluster_cols=FALSE))
}

plotCategory(select.samples.binCat,"BCcountNseq",c("totalLib","CNS100x_Str","CNS100x_Th","CNS100x_Ctx","CNS1
```


16
14
12
10
8
6
4
Chikungunya−virus
Neurotrophin
Lectin
Pseudorabies−virus
Herpes−B−virus
Herpes−simplex−virus−type−1
Herpes−simplex−virus−type−2
Horseradish−peroxidase
Neuron−related−prorein
Measles−virus
Vesicular−stomatitis−Indiana−virus
Enterovirus−71
Adeno−associated−virus
Canine−adenovirus−2
Poliovirus
Influenza−A−virus
Theilers−murine−encephalomyelitis−virus
Bornavirus
Japanese−encephalitis−virus
Tick−borne−encephalitis−virus
Neurotoxin
Rabies−virus
Human−immunodeficiency−virus−type−1
Lymphocytic−choriomeningitis−virus
West−Nile−virus
totalLib
CNS100x_Str
CNS100x_Th
CNS100x_Ctx
CNS100x_SN

```
plotCategory(select.samples.binCat,"BCcountNseq",c("CNS1000x_Str","CNS1000x_Th","CNS1000x_Ctx","CNS1000x_SN"
```

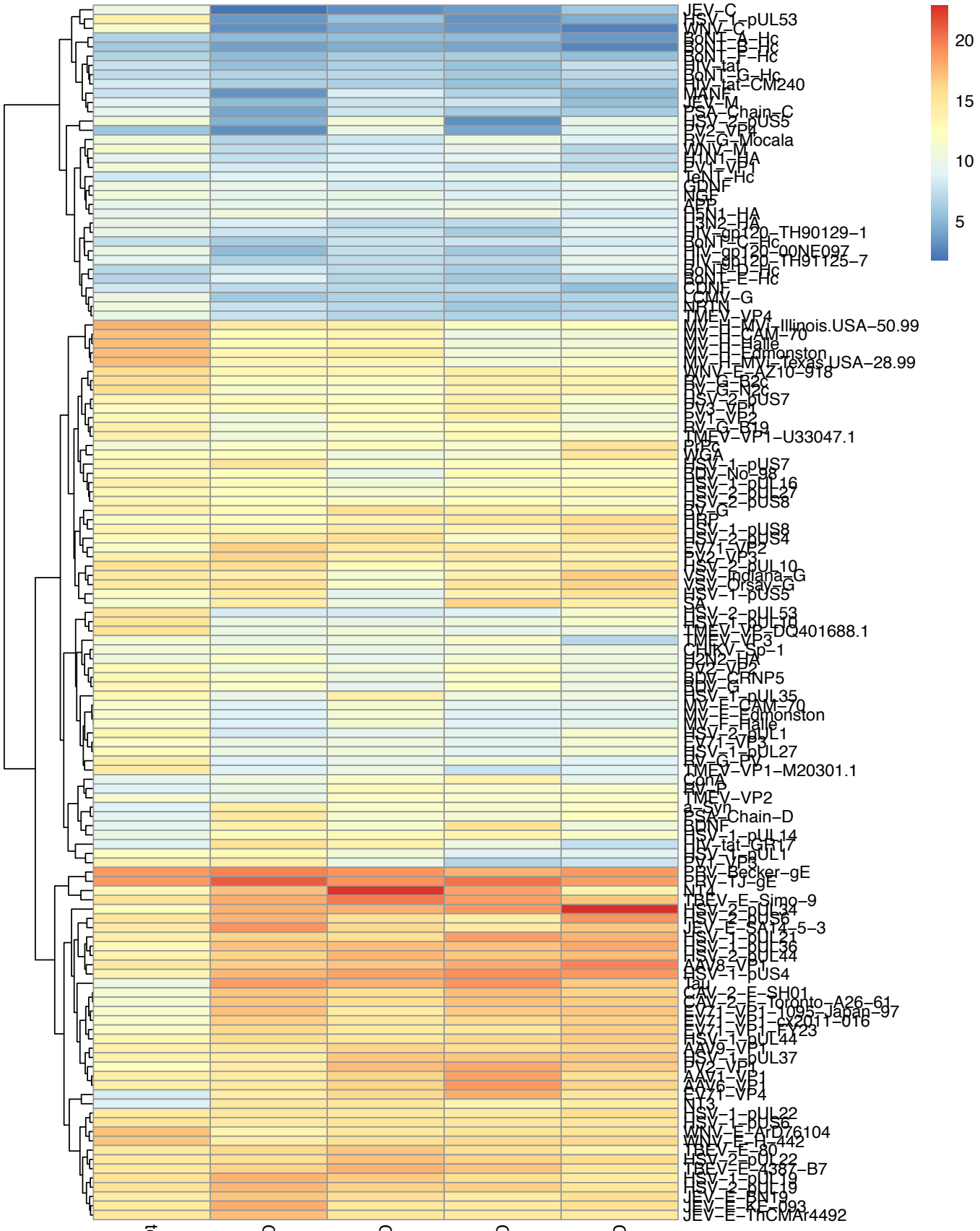

Measles−virus
Vesicular−stomatitis−Indiana−virus
Human−immunodeficiency−virus−type−1
Rabies−virus
West−Nile−virus
Enterovirus−71
Influenza−A−virus
Japanese−encephalitis−virus
Tick−borne−encephalitis−virus
Bornavirus
Neurotoxin
Adeno−associated−virus
Poliovirus
Chikungunya−virus
Theilers−murine−encephalomyelitis−virus
Herpes−B−virus
Neuron−related−prorein
Canine−adenovirus−2
Lymphocytic−choriomeningitis−virus
Horseradish−peroxidase
Herpes−simplex−virus−type−2
Lectin
Neurotrophin
Herpes−simplex−virus−type−1
Pseudorabies−virus
CNS1000x_Str
CNS1000x_Th
CNS1000x_Ctx
CNS1000x_SN
25
20
15
10
5

```
plotCategory(select.samples.binCat,"BCcountNseq",c("PrimN_100x","PrimN_1000x","293T_100x","293T_1000x"))
```

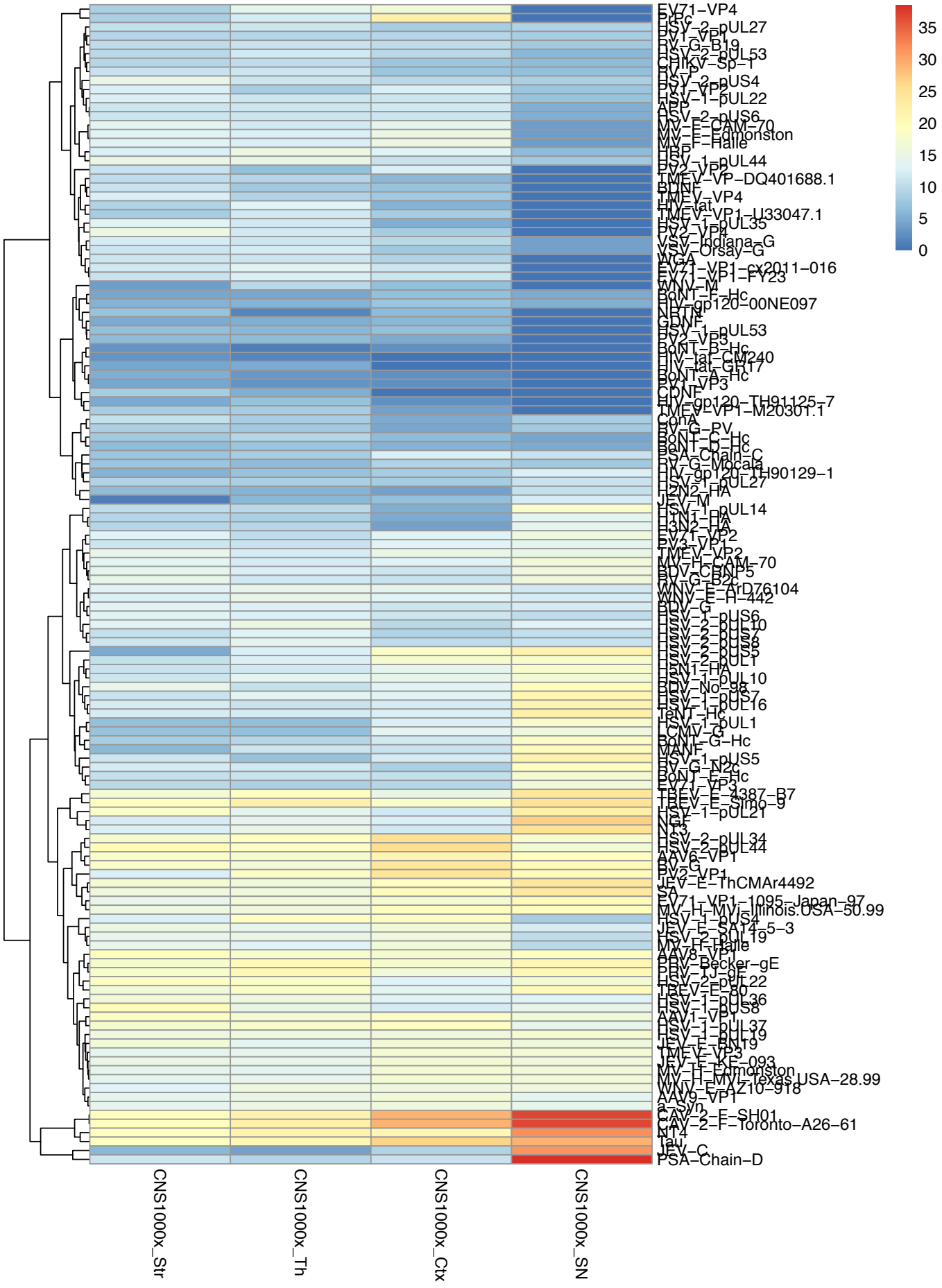

Tick−borne−encephalitis−virus
Bornavirus
Lymphocytic−choriomeningitis−virus
Canine−adenovirus−2
Horseradish−peroxidase
Chikungunya−virus
Pseudorabies−virus
Japanese−encephalitis−virus
Theilers−murine−encephalomyelitis−virus
Rabies−virus
West−Nile−virus
Human−immunodeficiency−virus−type−1
Measles−virus
Neurotrophin
Neurotoxin
Vesicular−stomatitis−Indiana−virus
Herpes−simplex−virus−type−2
Herpes−B−virus
Lectin
Enterovirus−71
Influenza−A−virus
Neuron−related−prorein
Herpes−simplex−virus−type−1
Adeno−associated−virus
Poliovirus
PrimN_100x
PrimN_1000x
293T_100x
293T_1000x
25
20
15
10
5
0

Plot Heatmaps split by GeneName

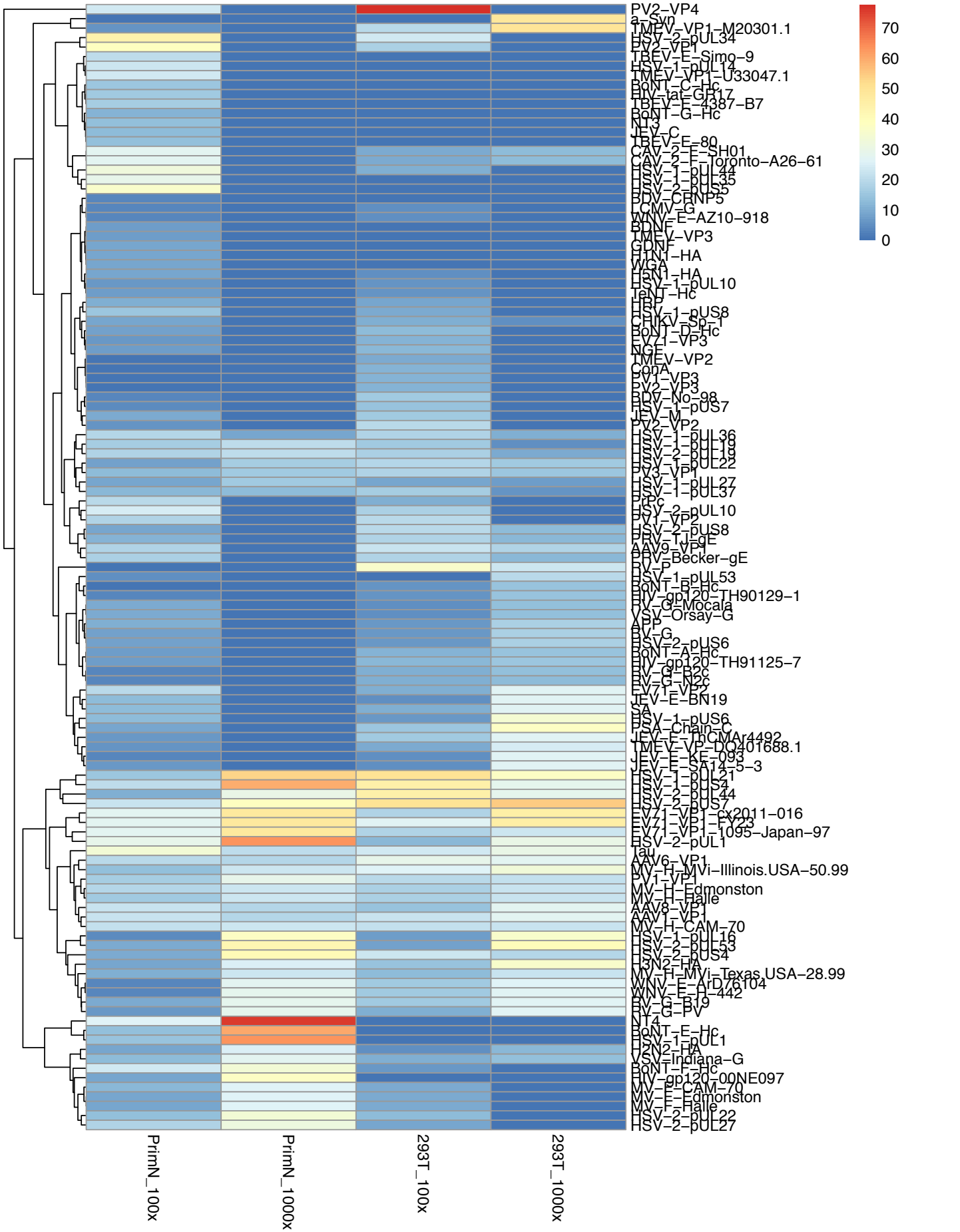
```
plotGene <- function(select.samples.table,plot.col,sample.select){
setkey(select.samples.table,Group)
select.samples.select <- select.samples.table[sample.select]
eval(parse(text=paste("setorder(select.samples.select,Group, -", plot.col,")", sep="")))
select.samples.matrix <- acast(select.samples.select, GeneName~Group, value.var=plot.col)
#Utilizes reshape 2 to make matrix for heatmap

select.samples.matrix[is.na(select.samples.matrix)] <- 0
select.samples.matrix <- select.samples.matrix[,sample.select]
return(pheatmap(select.samples.matrix, cluster_rows=TRUE, show_rownames=TRUE, cluster_cols=FALSE))
}

plotGene(select.samples.binGene,"BCcountNseq",c("totalLib","CNS100x_Str","CNS100x_Th","CNS100x_Ctx","CNS100x
```


20
15
10
5
totalLib
CNS100x_Str
CNS100x_Th
CNS100x_Ctx
CNS100x_SN

```
plotGene(select.samples.binGene,"BCcountNseq",c("CNS1000x_Str","CNS1000x_Th","CNS1000x_Ctx","CNS1000x_SN"))
```

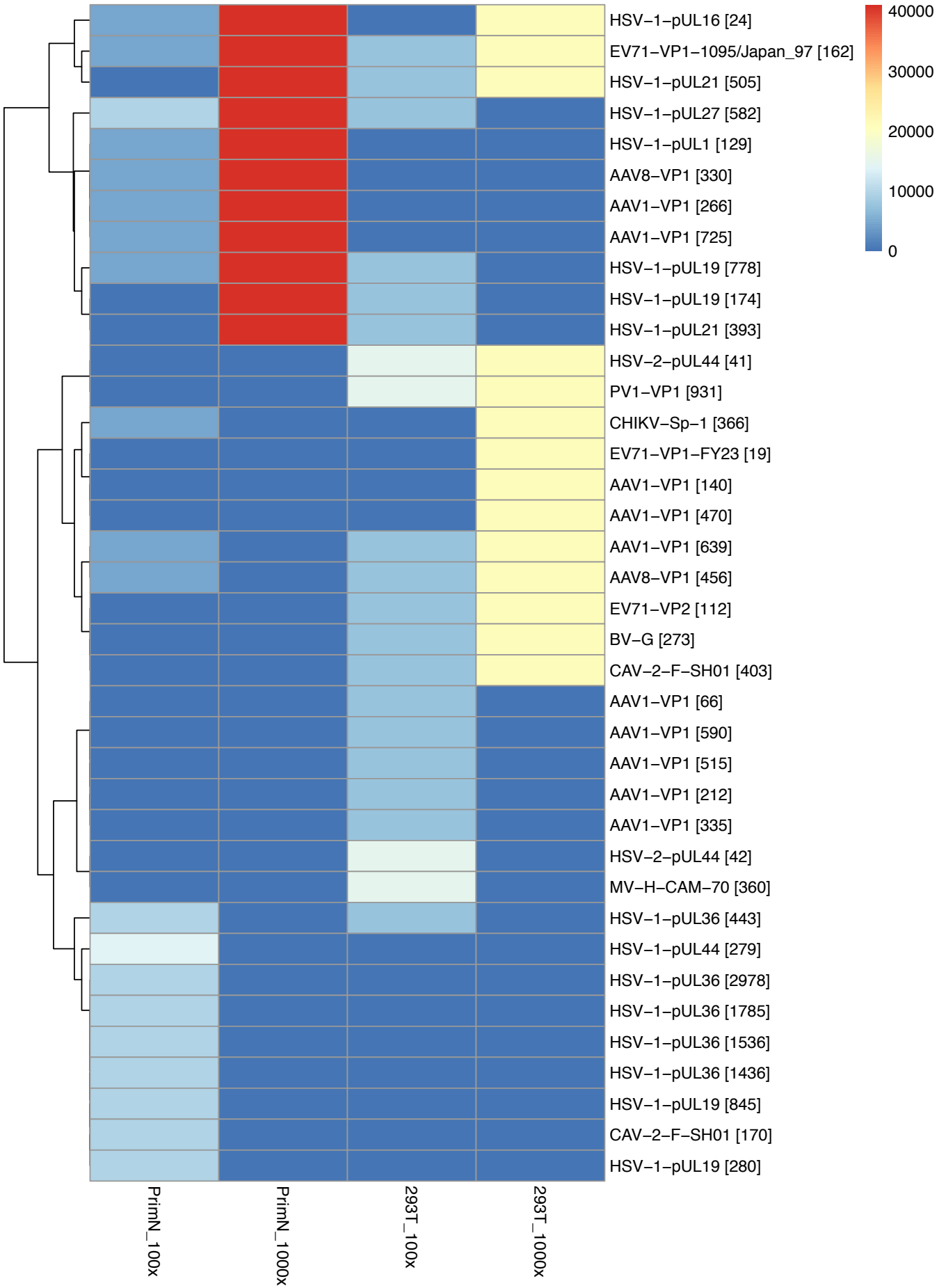

35
30
25
20
15
10
5
0
CNS1000x_Str
CNS1000x_Th
CNS1000x_Ctx
CNS1000x_SN

```
plotGene(select.samples.binGene,"BCcountNseq",c("PrimN_100x","PrimN_1000x","293T_100x","293T_1000x"))
```


PV2−VP4
a−Syn
TMEV−VP1−M20301.1
HSV−2−pUL34
PV2−VP1
TBEV−E−Simo−9
HSV−1−pUL14
TMEV−VP1−U33047.1
BoNT−C−Hc
HIV−tat−GR17
TBEV−E−4387−B7
BoNT−G−Hc
NT3
JEV−C
TBEV−E−80
CAV−2−E−SH01
CAV−2−F−Toronto−A26−61
HSV−1−pUL44
HSV−1−pUL35
HSV−2−pUS5
BDV−CRNP5
LCMV−G
WNV−E−AZ10−918
BDNF
TMEV−VP3
GDNF
H1N1−HA
WGA
H5N1−HA
HSV−1−pUL10
TeNT−Hc
HBP
HSV−1−pUS8
CHIKV−Sp−1
BoNT−D−Hc
EV71−VP3
NGF
TMEV−VP2
ConA
PV1−VP3
PV2−VP3
BDV−No−98
HSV−1−pUS7
JEV−M
PV2−VP2
HSV−1−pUL36
HSV−1−pUL18
HSV−2−pUL19
HSV−2−pUL22
PV3−VP1
HSV−1−pUL27
HSV−1−pUL37
PrPc
HSV−2−pUL10
PV1−VP2
HSV−2−pUS8
PRV−TJ−gE
AAV9−VP1
PRV−Becker−gE
PV−P
HSV−1−pUL53
BoNT−B−Hc
HIV−gp120−TH90129−1
RV−G−Mocala
VSV−Orsay−G
APP
RV−G
HSV−2−pUS6
BoNT−A−Hc
HIV−gp120−TH91125−7
RV−G−B2c
RV−G−N2c
EV71−VP2
JEV−E−BN19
SA
HSV−1−pUS6
PSA−Chain−C
JEV−E−ThCMAr4492
TMEV−VP−DQ401688.1
JEV−E−KE−093
JEV−E−SA14−5−3
HSV−1−pUL21
HSV−1−pUS4
HSV−2−pUL44
HSV−2−pUS7
EV71−VP1−cx2011−016
EV71−VP1−FY23
EV71−VP1−1095−Japan−97
HSV−2−pUL1
Tau
AAV6−VP1
MV−H−MVi−Illinois.USA−50.99
PV1−VP1
MV−H−Edmonston
MV−H−Halle
AAV8−VP1
AAV1−VP1
MV−H−CAM−70
HSV−1−pUL16
HSV−2−pUL53
HSV−2−pUS4
H3N2−HA
MV−H−MVi−Texas.USA−28.99
WNV−E−ArD7k104
WNV−E−H−442
RV−G−B19
RV−G−PV
NT4
BoNT−E−Hc
HSV−1−pUL1
H2N2−HA
VSV−Indiana−G
BoNT−F−Hc
HIV−gp120−00NE097
MV−E−CAM−70
MV−E−Edmonston
MV−E−Halle
HSV−2−pUL22
HSV−2−pUL27
PrimN_100x
PrimN_1000x
293T_100x
293T_1000x
70
60
50
40
30
20
10
0

Selection of top ten fragments per sample

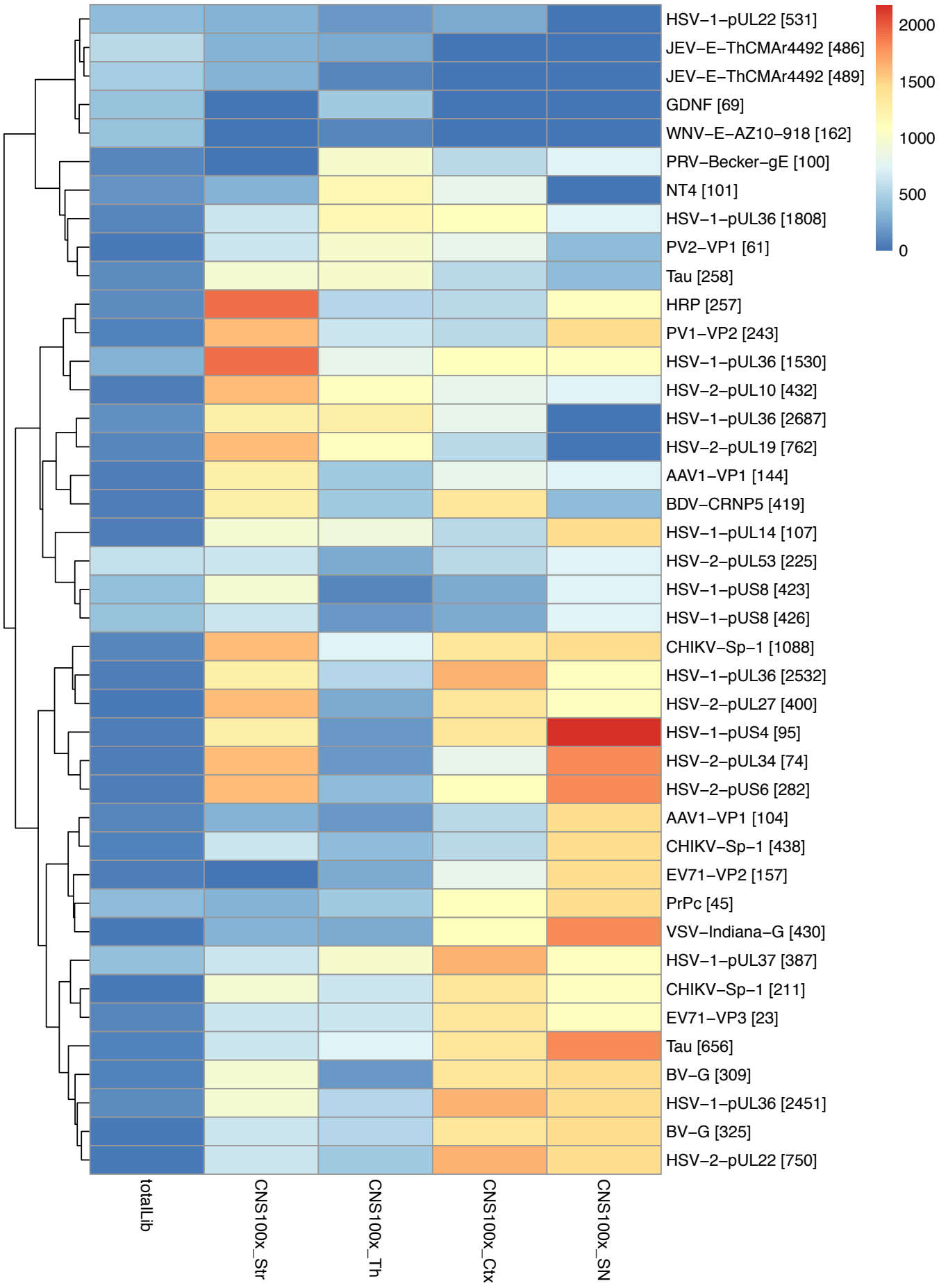
```
plotPos <- function(select.samples.table,plot.col,sample.select){
setkeyv(select.samples.table,"Group")
select.samples.select <- select.samples.table[sample.select]
eval(parse(text=paste("setorder(select.samples.select,Group, -", plot.col,")", sep="")))
select.samples.topTen <- select.samples.select[, head(.SD, 10), by=Group]
select.samples.out <- select.samples.select[select.samples.select$GeneAA %in% select.samples.topTen$GeneAA]
select.samples.out <- acast(select.samples.out, GeneAA~Group, value.var=plot.col) #Utilizes reshape 2 to mal
select.samples.out[is.na(select.samples.out)] <- 0
select.samples.out <- select.samples.out[,sample.select]
pheatmap(select.samples.out, cluster_rows=TRUE, show_rownames=TRUE, cluster_cols=FALSE)
select.samples.topTen[,c("totBC","seqlength","BCcountN","GeneAA","BCcountNseq"):=NULL]
return(knitr::kable(select.samples.topTen, format = "markdown"))
}

plotPos(select.samples.binPos,"BCcountN",c("PrimN_100x","PrimN_1000x","293T_100x","293T_1000x"))
```


HSV−1−pUL16 [24]
EV71−VP1−1095/Japan_97 [162]
HSV−1−pUL21 [505]
HSV−1−pUL27 [582]
HSV−1−pUL1 [129]
AAV8−VP1 [330]
AAV1−VP1 [266]
AAV1−VP1 [725]
HSV−1−pUL19 [778]
HSV−1−pUL19 [174]
HSV−1−pUL21 [393]
HSV−2−pUL44 [41]
PV1−VP1 [931]
CHIKV−Sp−1 [366]
EV71−VP1−FY23 [19]
AAV1−VP1 [140]
AAV1−VP1 [470]
AAV1−VP1 [639]
AAV8−VP1 [456]
EV71−VP2 [112]
BV−G [273]
CAV−2−F−SH01 [403]
AAV1−VP1 [66]
AAV1−VP1 [590]
AAV1−VP1 [515]
AAV1−VP1 [212]
AAV1−VP1 [335]
HSV−2−pUL44 [42]
MV−H−CAM−70 [360]
HSV−1−pUL36 [443]
HSV−1−pUL44 [279]
HSV−1−pUL36 [2978]
HSV−1−pUL36 [1785]
HSV−1−pUL36 [1536]
HSV−1−pUL36 [1436]
HSV−1−pUL19 [845]
CAV−2−F−SH01 [170]
HSV−1−pUL19 [280]
PrimN_100x
PrimN_1000x
293T_100x
293T_1000x
40000
30000
20000
10000
0

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches	BC
293T_1000x	AAV1-VP1	140	3.793435	1	1	14aaG4S	1.00	
293T_1000x	AAV1-VP1	470	3.793435	1	1	14aaA5	0.00	
293T_1000x	AAV1-VP1	639	3.793435	1	1	14aa	1.00	
293T_1000x	AAV8-VP1	456	3.793435	1	1	14aa	2.00	
293T_1000x	CAV-2-F-SH01	403	19.719528	1	1	14aa	0.00	
293T_1000x	CHIKV-Sp-1	366	3.622488	1	1	14aa	0.00	
293T_1000x	EV71-VP1-1095/Japan_97	162	12.490793	1	1	14aa	0.00	
293T_1000x	EV71-VP1-FY23	19	12.490793	1	1	14aa	0.00	
293T_1000x	EV71-VP2	112	12.490793	1	1	14aa	0.00	
293T_1000x	BV-G	273	4.562697	1	1	14aa	1.00	
293T_100x	HSV-2-pUL44	41	15.694712	2	1	14aa	0.50	
293T_100x	HSV-2-pUL44	42	15.694712	2	1	14aa	0.00	
293T_100x	MV-H-CAM-70	360	19.003300	2	1	14aa	0.50	
293T_100x	PV1-VP1	931	15.539818	2	1	14aa	3.50	
293T_100x	AAV1-VP1	66	15.059856	1	1	14aa	0.00	
293T_100x	AAV1-VP1	212	15.059856	1	1	14aaA5	0.00	
293T_100x	AAV1-VP1	335	15.059856	1	1	14aa	0.00	
293T_100x	AAV1-VP1	515	15.059856	1	1	14aa	7.00	
293T_100x	AAV1-VP1	590	15.059856	1	1	14aaA5	0.50	
293T_100x	AAV1-VP1	639	15.059856	1	1	14aa	0.00	
PrimN_1000x	AAV1-VP1	266	4.054469	1	1	14aaG4S	0.00	
PrimN_1000x	AAV1-VP1	725	4.054469	1	1	14aaA5	0.00	
PrimN_1000x	AAV8-VP1	330	4.054469	1	1	14aa	0.00	
PrimN_1000x	EV71-VP1-1095/Japan_97	162	3.864548	1	1	14aa	2.00	
PrimN_1000x	HSV-1-pUL1	129	10.780106	1	1	22aa	1.00	
PrimN_1000x	HSV-1-pUL16	24	10.780106	1	1	22aa	3.50	
PrimN_1000x	HSV-1-pUL19	174	10.780106	1	1	14aa	0.00	
PrimN_1000x	HSV-1-pUL19	778	10.780106	1	1	14aa	0.00	
PrimN_1000x	HSV-1-pUL21	393	10.780106	1	1	22aa	0.00	
PrimN_1000x	HSV-1-pUL21	505	10.780106	1	1	14aa	0.00	
PrimN_100x	HSV-1-pUL44	279	15.960704	3	1	14aa	0.00	
PrimN_100x	CAV-2-F-SH01	170	16.583186	2	1	14aa	1.00	
PrimN_100x	HSV-1-pUL19	280	15.960704	2	1	14aa	1.00	
PrimN_100x	HSV-1-pUL19	845	15.960704	2	1	14aa,14aaA5	0.00	
PrimN_100x	HSV-1-pUL27	582	15.960704	2	1	14aa	0.50	
PrimN_100x	HSV-1-pUL36	443	15.960704	2	1	14aa,14aaA5	0.50	
PrimN_100x	HSV-1-pUL36	1436	15.960704	2	1	14aa	0.25	
PrimN_100x	HSV-1-pUL36	1536	15.960704	2	1	14aa	0.00	
PrimN_100x	HSV-1-pUL36	1785	15.960704	2	1	14aa,22aa	0.00	

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches	BC
PrimN_100x	HSV-1-pUL36	2978	15.960704	2	1	14aa	2.25	

```
plotPos(select.samples.binPos,"BCcountN",c("totalLib","CNS100x_Str","CNS100x_Th","CNS100x_Ctx","CNS100x_SN"
```


HSV−1−pUL22 [531]
JEV−E−ThCMAr4492 [486]
JEV−E−ThCMAr4492 [489]
GDNF [69]
WNV−E−AZ10−918 [162]
PRV−Becker−gE [100]
NT4 [101]
HSV−1−pUL36 [1808]
PV2−VP1 [61]
Tau [258]
HRP [257]
PV1−VP2 [243]
HSV−1−pUL36 [1530]
HSV−2−pUL10 [432]
HSV−1−pUL36 [2687]
HSV−2−pUL19 [762]
AAV1−VP1 [144]
BDV−CRNP5 [419]
HSV−1−pUL14 [107]
HSV−2−pUL53 [225]
HSV−1−pUS8 [423]
HSV−1−pUS8 [426]
CHIKV−Sp−1 [1088]
HSV−1−pUL36 [2532]
HSV−2−pUL27 [400]
HSV−1−pUS4 [95]
HSV−2−pUL34 [74]
HSV−2−pUS6 [282]
AAV1−VP1 [104]
CHIKV−Sp−1 [438]
EV71−VP2 [157]
PrPc [45]
VSV−Indiana−G [430]
HSV−1−pUL37 [387]
CHIKV−Sp−1 [211]
EV71−VP3 [23]
Tau [656]
BV−G [309]
HSV−1−pUL36 [2451]
BV−G [325]
HSV−2−pUL22 [750]
totalLib
CNS100x_Str
CNS100x_Th
CNS100x_Ctx
CNS100x_SN
2000
1500
1000
500
0

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches
CNS100x_Ctx	HSV-1-pUL36	2451	6.764485	6	3	14aa	0.000
CNS100x_Ctx	HSV-1-pUL36	2532	6.764485	6	2	14aa,22aa	0.125
CNS100x_Ctx	HSV-1-pUL37	387	6.764485	6	2	14aa,22aa	0.500
CNS100x_Ctx	HSV-2-pUL22	750	7.015719	6	3	14aa,22aa	0.000
CNS100x_Ctx	BDV-CRNP5	419	6.943438	5	3	14aa,14aaG4S	0.000
CNS100x_Ctx	CHIKV-Sp-1	211	6.557930	5	2	14aa	0.000
CNS100x_Ctx	CHIKV-Sp-1	1088	6.557930	5	2	14aa,14aaA5	2.500
CNS100x_Ctx	EV71-VP3	23	7.696183	5	3	14aa,14aaA5	0.000
CNS100x_Ctx	BV-G	309	8.800267	5	2	14aa,22aa	0.250
CNS100x_Ctx	BV-G	325	8.800267	5	2	14aa	0.000
CNS100x_SN	HSV-1-pUS4	95	6.622249	6	3	14aa,14aaG4S	0.250
CNS100x_SN	HSV-2-pUL34	74	7.087182	5	3	14aa,14aaA5	0.750
CNS100x_SN	HSV-2-pUS6	282	7.087182	5	3	14aa	0.000
CNS100x_SN	Tau	656	6.452116	5	2	14aa,14aaA5,14aaG4S	0.000
CNS100x_SN	VSV-Indiana-G	430	5.260092	5	3	14aa,22aa	0.000
CNS100x_SN	AAV1-VP1	104	6.460376	4	3	14aa	0.625
CNS100x_SN	CHIKV-Sp-1	438	6.462385	4	2	14aa	2.500
CNS100x_SN	CHIKV-Sp-1	1088	6.462385	4	2	14aa,14aaA5	1.000
CNS100x_SN	EV71-VP2	157	6.458255	4	2	14aa	0.000
CNS100x_SN	BV-G	309	7.966180	4	2	14aa,22aa	0.000
CNS100x_Str	HSV-1-pUL36	1530	8.971415	6	3	22aa	0.000
CNS100x_Str	HRP	257	11.478122	6	3	14aa,14aaG4S	0.000
CNS100x_Str	CHIKV-Sp-1	1088	8.790423	5	2	14aa,14aaA5	0.125
CNS100x_Str	HSV-2-pUL10	432	9.005401	5	2	14aa	0.000
CNS100x_Str	HSV-2-pUL19	762	9.005401	5	2	14aa,22aa	0.000
CNS100x_Str	HSV-2-pUL27	400	9.005401	5	3	14aa	1.000
CNS100x_Str	HSV-2-pUL34	74	9.005401	5	3	14aa,14aaA5	0.500
CNS100x_Str	HSV-2-pUS6	282	9.005401	5	3	14aa,22aa	1.000
CNS100x_Str	PV1-VP2	243	8.497670	5	3	14aa	0.000
CNS100x_Str	AAV1-VP1	144	9.759704	4	2	22aa	0.000
CNS100x_Th	HSV-1-pUL36	2687	16.590961	14	3	14aa,14aaA5	0.000
CNS100x_Th	HSV-1-pUL36	1808	16.590961	13	3	14aa,14aaG4S	0.000
CNS100x_Th	NT4	101	17.830703	13	3	14aa,14aaA5,14aaG4S	0.500
CNS100x_Th	HSV-2-pUL10	432	16.450100	12	3	14aa	0.000
CNS100x_Th	HSV-2-pUL19	762	16.450100	12	3	14aa,22aa	0.000
CNS100x_Th	HSV-1-pUL37	387	16.590961	11	3	14aa,22aa	0.000
CNS100x_Th	Tau	258	17.749381	11	3	14aa	0.000
CNS100x_Th	PV2-VP1	61	15.425411	11	3	14aa	0.000
CNS100x_Th	PRV-Becker-gE	100	16.534930	11	3	14aa,14aaA5,14aaG4S	0.000

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches
CNS100x_Th	HSV-1-pUL14	107	16.590961	10	3	14aa	0.250
totalLib	HSV-2-pUL53	225	147.112080	603	1	14aa,22aa	0.500
totalLib	JEV-E-ThCMAr4492	486	121.229213	556	1	14aa,22aa	0.250
totalLib	JEV-E-ThCMAr4492	489	121.229213	463	1	14aa,22aa	0.000
totalLib	WNV-E-AZ10-918	162	132.096465	412	1	14aa,22aa	0.500
totalLib	HSV-1-pUS8	426	148.363716	402	1	14aa,22aa	0.500
totalLib	GDNF	69	115.597454	393	1	14aa,22aa	0.500
totalLib	HSV-1-pUL37	387	148.363716	388	1	14aa,22aa	1.250
totalLib	HSV-1-pUS8	423	148.363716	377	1	14aa,22aa	1.000
totalLib	HSV-1-pUL22	531	148.363716	363	1	14aa,22aa	0.250
totalLib	PrPc	45	115.234755	360	1	14aa,22aa	0.250

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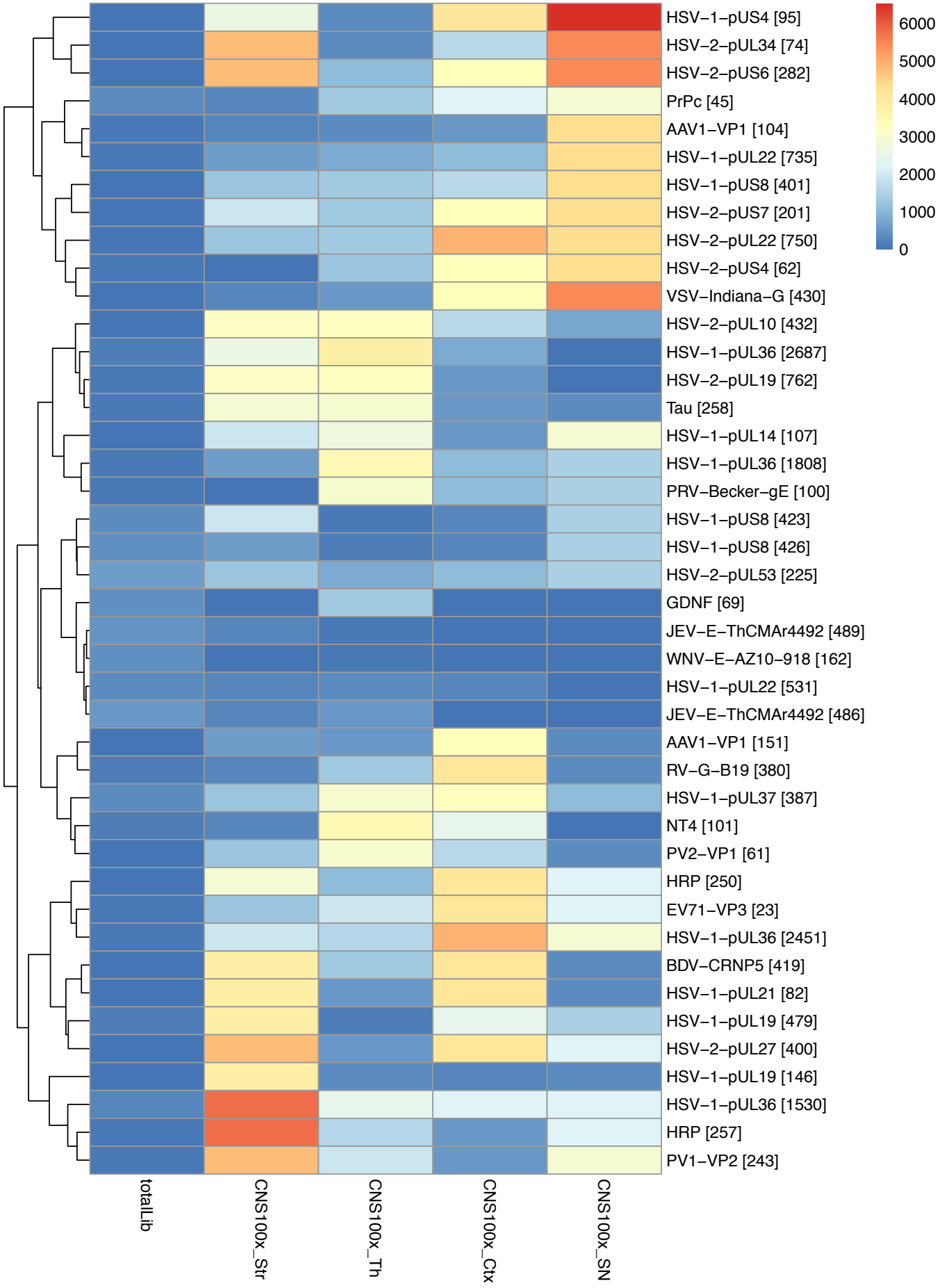
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HSV−2−pUL34 [74]
HSV−2−pUS6 [282]
PrPc [45]
AAV1−VP1 [104]
HSV−1−pUL22 [735]
HSV−1−pUS8 [401]
HSV−2−pUS7 [201]
HSV−2−pUL22 [750]
HSV−2−pUS4 [62]
VSV−Indiana−G [430]
HSV−2−pUL10 [432]
HSV−1−pUL36 [2687]
HSV−2−pUL19 [762]
Tau [258]
HSV−1−pUL14 [107]
HSV−1−pUL36 [1808]
PRV−Becker−gE [100]
HSV−1−pUS8 [423]
HSV−1−pUS8 [426]
HSV−2−pUL53 [225]
GDNF [69]
JEV−E−ThCMAr4492 [489]
WNV−E−AZ10−918 [162]
HSV−1−pUL22 [531]
JEV−E−ThCMAr4492 [486]
AAV1−VP1 [151]
RV−G−B19 [380]
HSV−1−pUL37 [387]
NT4 [101]
PV2−VP1 [61]
HRP [250]
EV71−VP3 [23]
HSV−1−pUL36 [2451]
BDV−CRNP5 [419]
HSV−1−pUL21 [82]
HSV−1−pUL19 [479]
HSV−2−pUL27 [400]
HSV−1−pUL19 [146]
HSV−1−pUL36 [1530]
HRP [257]
PV1−VP2 [243]
totalLib
CNS100x_Str
CNS100x_Th
CNS100x_Ctx
CNS100x_SN
6000
5000
4000
3000
2000
1000
0

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches
CNS100x_Ctx	HSV-1-pUL36	2451	6.764485	6	3	14aa	0.000
CNS100x_Ctx	HSV-2-pUL22	750	7.015719	6	3	14aa,22aa	0.000
CNS100x_Ctx	BDV-CRNP5	419	6.943438	5	3	14aa,14aaG4S	0.000
CNS100x_Ctx	EV71-VP3	23	7.696183	5	3	14aa,14aaA5	0.000
CNS100x_Ctx	HSV-1-pUL21	82	6.764485	5	3	14aa	0.000
CNS100x_Ctx	HSV-1-pUS4	95	6.764485	5	3	14aa,14aaG4S	0.500
CNS100x_Ctx	HSV-2-pUL27	400	7.015719	5	3	14aa	1.000
CNS100x_Ctx	HRP	250	6.682046	5	3	14aa	0.000
CNS100x_Ctx	RV-G-B19	380	6.440835	5	3	14aaA5,14aaG4S	0.500
CNS100x_Ctx	AAV1-VP1	151	7.169508	4	3	14aa	0.000
CNS100x_SN	HSV-1-pUS4	95	6.622249	6	3	14aa,14aaG4S	0.250
CNS100x_SN	HSV-2-pUL34	74	7.087182	5	3	14aa,14aaA5	0.750
CNS100x_SN	HSV-2-pUS6	282	7.087182	5	3	14aa	0.000
CNS100x_SN	VSV-Indiana-G	430	5.260092	5	3	14aa,22aa	0.000
CNS100x_SN	AAV1-VP1	104	6.460376	4	3	14aa	0.625
CNS100x_SN	HSV-1-pUL22	735	6.622249	4	3	14aa	0.250
CNS100x_SN	HSV-1-pUS8	401	6.622249	4	3	14aaA5	0.000
CNS100x_SN	HSV-2-pUL22	750	7.087182	4	3	14aa	0.500
CNS100x_SN	HSV-2-pUS4	62	7.087182	4	3	14aa	0.250
CNS100x_SN	HSV-2-pUS7	201	7.087182	4	3	14aa	0.000
CNS100x_Str	HSV-1-pUL36	1530	8.971415	6	3	22aa	0.000
CNS100x_Str	HRP	257	11.478122	6	3	14aa,14aaG4S	0.000
CNS100x_Str	HSV-2-pUL27	400	9.005401	5	3	14aa	1.000
CNS100x_Str	HSV-2-pUL34	74	9.005401	5	3	14aa,14aaA5	0.500
CNS100x_Str	HSV-2-pUS6	282	9.005401	5	3	14aa,22aa	1.000
CNS100x_Str	PV1-VP2	243	8.497670	5	3	14aa	0.000
CNS100x_Str	BDV-CRNP5	419	10.235977	4	3	14aa,14aaG4S	0.000
CNS100x_Str	HSV-1-pUL19	146	8.971415	4	3	14aa,14aaG4S	1.625
CNS100x_Str	HSV-1-pUL19	479	8.971415	4	3	14aa,14aaG4S	0.000
CNS100x_Str	HSV-1-pUL21	82	8.971415	4	3	14aa	0.500
CNS100x_Th	HSV-1-pUL36	2687	16.590961	14	3	14aa,14aaA5	0.000
CNS100x_Th	HSV-1-pUL36	1808	16.590961	13	3	14aa,14aaG4S	0.000
CNS100x_Th	NT4	101	17.830703	13	3	14aa,14aaA5,14aaG4S	0.500
CNS100x_Th	HSV-2-pUL10	432	16.450100	12	3	14aa	0.000
CNS100x_Th	HSV-2-pUL19	762	16.450100	12	3	14aa,22aa	0.000
CNS100x_Th	HSV-1-pUL37	387	16.590961	11	3	14aa,22aa	0.000
CNS100x_Th	Tau	258	17.749381	11	3	14aa	0.000
CNS100x_Th	PV2-VP1	61	15.425411	11	3	14aa	0.000
CNS100x_Th	PRV-Becker-gE	100	16.534930	11	3	14aa,14aaA5,14aaG4S	0.000

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches
CNS100x_Th	HSV-1-pUL14	107	16.590961	10	3	14aa	0.250
totalLib	HSV-2-pUL53	225	147.112080	603	1	14aa,22aa	0.500
totalLib	JEV-E-ThCMAr4492	486	121.229213	556	1	14aa,22aa	0.250
totalLib	JEV-E-ThCMAr4492	489	121.229213	463	1	14aa,22aa	0.000
totalLib	WNV-E-AZ10-918	162	132.096465	412	1	14aa,22aa	0.500
totalLib	HSV-1-pUS8	426	148.363716	402	1	14aa,22aa	0.500
totalLib	GDNF	69	115.597454	393	1	14aa,22aa	0.500
totalLib	HSV-1-pUL37	387	148.363716	388	1	14aa,22aa	1.250
totalLib	HSV-1-pUS8	423	148.363716	377	1	14aa,22aa	1.000
totalLib	HSV-1-pUL22	531	148.363716	363	1	14aa,22aa	0.250
totalLib	PrPc	45	115.234755	360	1	14aa,22aa	0.250

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plotPos(select.samples.binPos,"BCcountN",c("CNS1000x_Str","CNS1000x_Th","CNS1000x_Ctx","CNS1000x_SN"))
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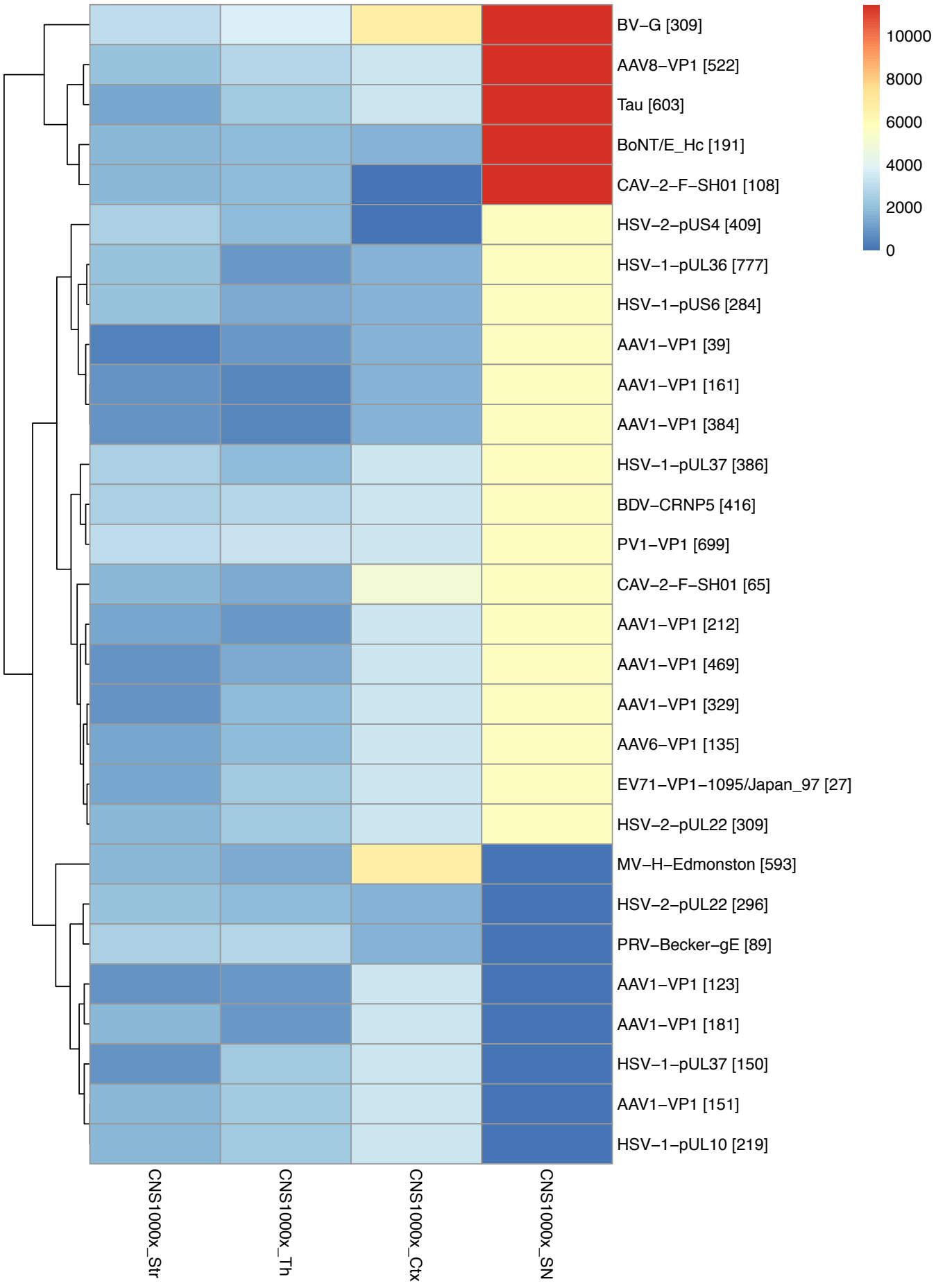
BV–G [309]
AAV8–VP1 [522]
Tau [603]
BoNT/E_Hc [191]
CAV–2–F–SH01 [108]
HSV–2–pUS4 [409]
HSV–1–pUL36 [777]
HSV–1–pUS6 [284]
AAV1–VP1 [39]
AAV1–VP1 [161]
AAV1–VP1 [384]
HSV–1–pUL37 [386]
BDV–CRNP5 [416]
PV1–VP1 [699]
CAV–2–F–SH01 [65]
AAV1–VP1 [212]
AAV1–VP1 [469]
AAV1–VP1 [329]
AAV6–VP1 [135]
EV71–VP1–1095/Japan_97 [27]
HSV–2–pUL22 [309]
MV–H–Edmonston [593]
HSV–2–pUL22 [296]
PRV–Becker–gE [89]
AAV1–VP1 [123]
AAV1–VP1 [181]
HSV–1–pUL37 [150]
AAV1–VP1 [151]
HSV–1–pUL10 [219]
CNS1000x_Str
CNS1000x_Th
CNS1000x_Ctx
CNS1000x_SN
10000
8000
6000
4000
2000
0

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches	E
CNS1000x_Ctx	BV-G	309	5.866275	4	2	14aa	0.500	
CNS1000x_Ctx	MV-H-Edmonston	593	5.397283	4	2	14aa,14aaA5	0.375	
CNS1000x_Ctx	CAV-2-F-SH01	65	6.278143	3	2	14aaA5	1.000	
CNS1000x_Ctx	AAV1-VP1	123	8.399157	2	2	14aa	0.000	
CNS1000x_Ctx	AAV1-VP1	151	8.399157	2	2	14aa	0.500	
CNS1000x_Ctx	AAV1-VP1	181	8.399157	2	2	14aa	0.000	
CNS1000x_Ctx	AAV1-VP1	212	8.399157	2	2	14aaA5	0.000	
CNS1000x_Ctx	AAV1-VP1	329	8.399157	2	2	14aa	1.250	
CNS1000x_Ctx	AAV1-VP1	469	8.399157	2	2	14aa	0.000	
CNS1000x_Ctx	AAV6-VP1	135	8.399157	2	2	14aa	0.500	
CNS1000x_SN	AAV8-VP1	522	5.808903	2	1	14aa	0.000	
CNS1000x_SN	CAV-2-F-SH01	108	7.265575	2	1	14aa	0.000	
CNS1000x_SN	BV-G	309	6.566840	2	1	14aa	0.500	
CNS1000x_SN	Tau	603	4.875844	2	1	14aa,22aa	0.000	
CNS1000x_SN	BoNT/E_Hc	191	7.920536	2	2	14aa	0.500	
CNS1000x_SN	AAV1-VP1	39	5.808903	1	1	14aa	0.000	
CNS1000x_SN	AAV1-VP1	161	5.808903	1	1	14aa	0.000	
CNS1000x_SN	AAV1-VP1	212	5.808903	1	1	14aaA5	0.000	
CNS1000x_SN	AAV1-VP1	329	5.808903	1	1	14aa	0.000	
CNS1000x_SN	AAV1-VP1	384	5.808903	1	1	14aa	2.000	
CNS1000x_Str	BV-G	309	9.321071	7	3	14aa	1.000	
CNS1000x_Str	PV1-VP1	699	5.781098	7	4	14aa,22aa	0.000	
CNS1000x_Str	BDV-CRNP5	416	7.189508	6	4	14aaA5	0.000	
CNS1000x_Str	HSV-1-pUL37	386	6.236153	6	4	14aa,14aaG4S	0.000	
CNS1000x_Str	HSV-2-pUS4	409	5.981587	6	4	14aa	0.000	
CNS1000x_Str	PRV-Becker-gE	89	6.322660	6	4	14aa,22aa	0.000	
CNS1000x_Str	AAV8-VP1	522	8.164010	5	3	14aa	0.000	
CNS1000x_Str	HSV-1-pUL36	777	6.236153	5	4	14aa	0.000	
CNS1000x_Str	HSV-1-pUS6	284	6.236153	5	4	14aa,14aaA5	0.125	
CNS1000x_Str	HSV-2-pUL22	296	5.981587	5	3	14aa,14aaA5	0.125	
CNS1000x_Th	BV-G	309	25.314057	8	4	14aa	0.000	
CNS1000x_Th	PV1-VP1	699	15.265347	7	4	14aa,22aa	0.000	
CNS1000x_Th	AAV8-VP1	522	16.541045	6	4	14aa	0.500	
CNS1000x_Th	BDV-CRNP5	416	15.145850	6	4	14aaA5	0.000	
CNS1000x_Th	PRV-Becker-gE	89	17.024871	6	4	14aa,22aa	0.875	
CNS1000x_Th	AAV1-VP1	151	16.541045	5	4	14aa	0.500	
CNS1000x_Th	EV71-VP1-1095/Japan_97	27	14.101092	5	4	14aa	0.000	
CNS1000x_Th	HSV-1-pUL10	219	15.049748	5	3	14aa	0.000	
CNS1000x_Th	HSV-1-pUL37	150	15.049748	5	4	22aa	0.000	

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches	E
CNS1000x_Th	HSV-2-pUL22	309	14.677593	5	3	14aa	0.500	

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plotPos(select.samples.binPos,"BCcountNanim",c("CNS1000x_Str","CNS1000x_Th","CNS1000x_Ctx","CNS1000x_SN"))
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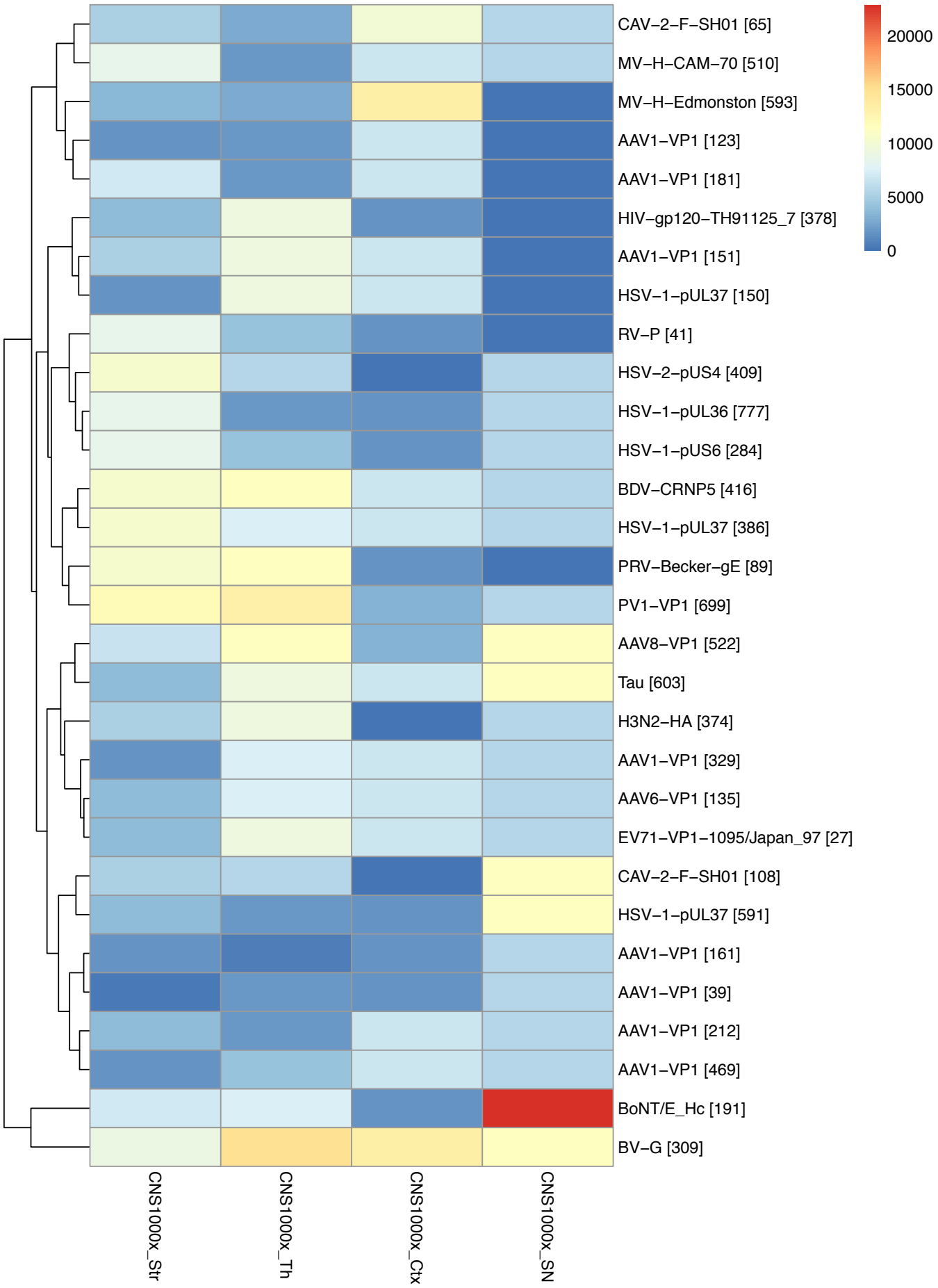
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MV−H−CAM−70 [510]
MV−H−Edmonston [593]
AAV1−VP1 [123]
AAV1−VP1 [181]
HIV−gp120−TH91125_7 [378]
AAV1−VP1 [151]
HSV−1−pUL37 [150]
RV−P [41]
HSV−2−pUS4 [409]
HSV−1−pUL36 [777]
HSV−1−pUS6 [284]
BDV−CRNP5 [416]
HSV−1−pUL37 [386]
PRV−Becker−gE [89]
PV1−VP1 [699]
AAV8−VP1 [522]
Tau [603]
H3N2−HA [374]
AAV1−VP1 [329]
AAV6−VP1 [135]
EV71−VP1−1095/Japan_97 [27]
CAV−2−F−SH01 [108]
HSV−1−pUL37 [591]
AAV1−VP1 [161]
AAV1−VP1 [39]
AAV1−VP1 [212]
AAV1−VP1 [469]
BoNT/E_Hc [191]
BV−G [309]
20000
15000
10000
5000
0
CNS1000x_Str
CNS1000x_Th
CNS1000x_Ctx
CNS1000x_SN

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches	E
CNS1000x_Ctx	BV-G	309	5.866275	4	2	14aa	0.500	
CNS1000x_Ctx	MV-H-Edmonston	593	5.397283	4	2	14aa,14aaA5	0.375	
CNS1000x_Ctx	CAV-2-F-SH01	65	6.278143	3	2	14aaA5	1.000	
CNS1000x_Ctx	AAV1-VP1	123	8.399157	2	2	14aa	0.000	
CNS1000x_Ctx	AAV1-VP1	151	8.399157	2	2	14aa	0.500	
CNS1000x_Ctx	AAV1-VP1	181	8.399157	2	2	14aa	0.000	
CNS1000x_Ctx	AAV1-VP1	212	8.399157	2	2	14aaA5	0.000	
CNS1000x_Ctx	AAV1-VP1	329	8.399157	2	2	14aa	1.250	
CNS1000x_Ctx	AAV1-VP1	469	8.399157	2	2	14aa	0.000	
CNS1000x_Ctx	AAV6-VP1	135	8.399157	2	2	14aa	0.500	
CNS1000x_SN	BoNT/E_Hc	191	7.920536	2	2	14aa	0.500	
CNS1000x_SN	AAV8-VP1	522	5.808903	2	1	14aa	0.000	
CNS1000x_SN	CAV-2-F-SH01	108	7.265575	2	1	14aa	0.000	
CNS1000x_SN	BV-G	309	6.566840	2	1	14aa	0.500	
CNS1000x_SN	HSV-1-pUL37	591	4.918278	1	2	22aa	0.000	
CNS1000x_SN	Tau	603	4.875844	2	1	14aa,22aa	0.000	
CNS1000x_SN	AAV1-VP1	39	5.808903	1	1	14aa	0.000	
CNS1000x_SN	AAV1-VP1	161	5.808903	1	1	14aa	0.000	
CNS1000x_SN	AAV1-VP1	212	5.808903	1	1	14aaA5	0.000	
CNS1000x_SN	AAV1-VP1	329	5.808903	1	1	14aa	0.000	
CNS1000x_Str	PV1-VP1	699	5.781098	7	4	14aa,22aa	0.000	
CNS1000x_Str	BDV-CRNP5	416	7.189508	6	4	14aaA5	0.000	
CNS1000x_Str	HSV-1-pUL37	386	6.236153	6	4	14aa,14aaG4S	0.000	
CNS1000x_Str	HSV-2-pUS4	409	5.981587	6	4	14aa	0.000	
CNS1000x_Str	PRV-Becker-gE	89	6.322660	6	4	14aa,22aa	0.000	
CNS1000x_Str	BV-G	309	9.321071	7	3	14aa	1.000	
CNS1000x_Str	HSV-1-pUL36	777	6.236153	5	4	14aa	0.000	
CNS1000x_Str	HSV-1-pUS6	284	6.236153	5	4	14aa,14aaA5	0.125	
CNS1000x_Str	MV-H-CAM-70	510	5.781607	5	4	14aa,22aa	0.250	
CNS1000x_Str	RV-P	41	5.264432	5	4	14aaA5	0.000	
CNS1000x_Th	BV-G	309	25.314057	8	4	14aa	0.000	
CNS1000x_Th	PV1-VP1	699	15.265347	7	4	14aa,22aa	0.000	
CNS1000x_Th	AAV8-VP1	522	16.541045	6	4	14aa	0.500	
CNS1000x_Th	BDV-CRNP5	416	15.145850	6	4	14aaA5	0.000	
CNS1000x_Th	PRV-Becker-gE	89	17.024871	6	4	14aa,22aa	0.875	
CNS1000x_Th	AAV1-VP1	151	16.541045	5	4	14aa	0.500	
CNS1000x_Th	EV71-VP1-1095/Japan_97	27	14.101092	5	4	14aa	0.000	
CNS1000x_Th	HSV-1-pUL37	150	15.049748	5	4	22aa	0.000	
CNS1000x_Th	HIV-gp120-TH91125_7	378	12.531731	5	4	22aa	0.000	

Group	GeneName	AA	NormCount	BCcount	AnimalCount	mainStruct	mismatches	E
CNS1000x_Th	H3N2-HA	374	14.171908	5	4	14aa	1.000	

```
devtools::session_info()
```

```
Session info -------------------------------------------------------------

 setting  value
 version  R version 3.2.3 (2015-12-10)
 system   x86_64, linux-gnu
 ui       X11
 language en_US:en
 collate  en_US.UTF-8
 tz       <NA>
 date     2016-01-13

Packages -----------------------------------------------------------------

 package              * version     date       source
 acepack                1.3-3.3     2014-11-24 CRAN (R 3.2.0)
 annotate               1.48.0      2016-01-07 Bioconductor
 AnnotationDbi          1.32.0      2015-11-26 Bioconductor
 Biobase              * 2.30.0      2015-11-26 Bioconductor
 BiocGenerics         * 0.16.1      2015-11-26 Bioconductor
 BiocInstaller          1.20.1      2015-11-26 Bioconductor
 BiocParallel           1.4.0       2015-11-26 Bioconductor
 biomaRt                2.26.1      2015-11-26 Bioconductor
 Biostrings           * 2.38.2      2015-11-26 Bioconductor
 biovizBase             1.18.0      2015-11-26 Bioconductor
 bitops                 1.0-6       2013-08-17 CRAN (R 3.2.0)
 BSgenome               1.38.0      2015-11-26 Bioconductor
 chron                  2.3-47      2015-06-24 CRAN (R 3.2.2)
 cluster                2.0.3       2015-07-21 CRAN (R 3.2.2)
 codetools              0.2-14      2015-07-15 CRAN (R 3.2.2)
 colorspace             1.2-6       2015-03-11 CRAN (R 3.2.0)
 data.table           * 1.9.6       2015-09-19 CRAN (R 3.2.2)
 DBI                    0.3.1       2014-09-24 CRAN (R 3.2.0)
 DESeq2               * 1.10.1      2016-01-07 Bioconductor
 devtools             * 1.9.1       2015-09-11 CRAN (R 3.2.2)
 dichromat              2.0-0       2013-01-24 CRAN (R 3.2.0)
 digest                 0.6.8       2014-12-31 CRAN (R 3.2.0)
 doParallel           * 1.0.10      2015-10-14 CRAN (R 3.2.2)
 evaluate               0.8         2015-09-18 CRAN (R 3.2.2)
 foreach              * 1.4.3       2015-10-13 CRAN (R 3.2.2)
 foreign                0.8-66      2015-08-19 CRAN (R 3.2.2)
 formatR                1.2.1       2015-09-18 CRAN (R 3.2.2)
 Formula                1.2-1       2015-04-07 CRAN (R 3.2.0)
 futile.logger          1.4.1       2015-04-20 CRAN (R 3.2.0)
 futile.options         1.0.0       2010-04-06 CRAN (R 3.2.0)
 genefilter             1.52.0      2016-01-07 Bioconductor
 geneplotter            1.48.0      2016-01-07 Bioconductor
 GenomeInfoDb         * 1.6.1       2015-11-26 Bioconductor
 GenomicAlignments    * 1.6.1       2015-11-26 Bioconductor
 GenomicFeatures        1.22.5      2015-11-26 Bioconductor
```

```
GenomicRanges        * 1.22.1      2015-11-26 Bioconductor
GGally                 0.5.0       2014-12-02 CRAN (R 3.2.0)
ggbio                * 1.18.1      2015-11-26 Bioconductor
ggplot2              * 1.0.1       2015-03-17 CRAN (R 3.2.0)
graph                  1.48.0      2015-11-26 Bioconductor
gridExtra              2.0.0       2015-07-14 CRAN (R 3.2.2)
gtable                 0.1.2       2012-12-05 CRAN (R 3.2.0)
highr                  0.5.1       2015-09-18 CRAN (R 3.2.2)
Hmisc                  3.17-0      2015-09-21 CRAN (R 3.2.2)
htmltools              0.2.6       2014-09-08 CRAN (R 3.2.0)
IRanges              * 2.4.4       2015-11-26 Bioconductor
iterators            * 1.0.8       2015-10-13 CRAN (R 3.2.2)
knitr                * 1.11        2015-08-14 CRAN (R 3.2.2)
lambda.r               1.1.7       2015-03-20 CRAN (R 3.2.0)
lattice                0.20-33     2015-07-14 CRAN (R 3.2.2)
latticeExtra           0.6-26      2013-08-15 CRAN (R 3.2.0)
locfit                 1.5-9.1     2013-04-20 CRAN (R 3.2.2)
magrittr               1.5         2014-11-22 CRAN (R 3.2.2)
MASS                   7.3-45      2015-11-10 CRAN (R 3.2.2)
memoise                0.2.1       2014-04-22 CRAN (R 3.2.2)
munsell                0.4.2       2013-07-11 CRAN (R 3.2.0)
nnet                   7.3-11      2015-08-30 CRAN (R 3.2.2)
OrganismDbi            1.12.0      2015-11-26 Bioconductor
pheatmap             * 1.0.8       2015-12-11 CRAN (R 3.2.3)
plyr                 * 1.8.3       2015-06-12 CRAN (R 3.2.2)
proto                  0.3-10      2012-12-22 CRAN (R 3.2.0)
RBGL                   1.46.0      2015-11-26 Bioconductor
RColorBrewer           1.1-2       2014-12-07 CRAN (R 3.2.0)
Rcpp                 * 0.12.2      2015-11-15 CRAN (R 3.2.2)
RcppArmadillo        * 0.6.400.2.2 2015-12-16 CRAN (R 3.2.3)
RCurl                  1.95-4.7    2015-06-30 CRAN (R 3.2.2)
reshape                0.8.5       2014-04-23 CRAN (R 3.2.0)
reshape2             * 1.4.1       2014-12-06 CRAN (R 3.2.0)
rmarkdown              0.8.1       2015-10-10 CRAN (R 3.2.2)
rpart                  4.1-10      2015-06-29 CRAN (R 3.2.2)
Rsamtools            * 1.22.0      2015-11-26 Bioconductor
RSQLite                1.0.0       2014-10-25 CRAN (R 3.2.0)
rtracklayer            1.30.1      2015-11-26 Bioconductor
S4Vectors            * 0.8.3       2015-11-26 Bioconductor
scales                 0.3.0       2015-08-25 CRAN (R 3.2.2)
stringi                1.0-1       2015-10-22 CRAN (R 3.2.2)
stringr                1.0.0       2015-04-30 CRAN (R 3.2.2)
SummarizedExperiment * 1.0.1       2015-11-26 Bioconductor
survival               2.38-3      2015-07-02 CRAN (R 3.2.2)
VariantAnnotation      1.16.3      2015-11-26 Bioconductor
XML                    3.98-1.3    2015-06-30 CRAN (R 3.2.2)
xtable                 1.8-0       2015-11-02 CRAN (R 3.2.2)
XVector              * 0.10.0      2015-11-26 Bioconductor
yaml                   2.1.13      2014-06-12 CRAN (R 3.2.0)
zlibbioc               1.16.0      2015-11-26 Bioconductor
```