T-cell repertoire annotation and motif discovery

A RepSeq data analysis tutorial in R

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RepSeq sample annotation

Here is the layout of our experiment, datasets were selected from *Emerson et al. Nat Genet 2017*.

Samples:

```
(B35+)       HIP02877  A*26 A*33 B*14 B*35  CMV-
(CMV+)       HIP13994  A*02 A*02 B*07 B*44  CMV+
```

Controls:

```
(Control-1)  HIP03484  A*02 A*02 B*07 B*58  CMV-
(Control-2)  HIP03592  A*02 A*32 B*07 B*39  CMV-
(Control-3)  HIP04532  A*02 A*24 B*07 B*51  CMV-
(Control-4)  HIP04576  A*02 A*30 B*07 B*18  CMV-
```

Compute some basic statistics using VDJtools.

```
run_java("vdjtools",
         "CalcBasicStats data/control.txt.gz data/CMV+.txt.gz data/B35+.txt.gz output/",
         T)
```

Number of reads and clonotypes per sample:

```
df.stats <- fread("output/basicstats.txt")
df.stats
```

```
##    sample_id metadata_blank    count diversity mean_frequency
## 1:   control              . 10881045    913905   1.094206e-06
## 2:      CMV+              .  3819906    187639   5.329382e-06
## 3:      B35+              .   899992     63737   1.568947e-05
##    geomean_frequency nc_diversity nc_frequency mean_cdr3nt_length
## 1:      6.754538e-07            0    0.0000000           43.16485
## 2:      2.593038e-06        33513    0.1621477           44.70757
## 3:      7.764453e-06            0    0.0000000           43.34150
##    mean_insert_size mean_ndn_size convergence
## 1:         3.079917      11.25928    1.112407
## 2:         4.183891      12.30924    1.036891
## 3:         2.717946      10.46588    1.027992
```

Annotate samples using VDJmatch. The following arguments are used:

- `match` runs routine that matches samples against VDJdb
- `-S human` sets species
- `-R TRB` sets receptor chain
- `-O 1,0,1` sets the search scope - number of substitutions, indels and total number of mutations. Here we'll just allow a single substitution. Note that allowing indels can make results quite messy (need to use correct scoring with `-A` argument)
- `--min-epi-size 30` will select VDJdb epitopes that have at least 30 unique TCR records

```
run_java("vdjmatch",
         "match -S human -R TRB -O 1,0,1 --min-epi-size 30 data/control.txt.gz data/CMV+.txt.gz data/B3
         T)
```

Lets explore annotation results. Load and quality-filter VDJdb annotations

```
# Read in data
list("control", "CMV+", "B35+") %>%
  lapply(function(x)
    "output/vdjdb.{x}.txt" %>%
      str_glue() %>%
      fread() %>%
      mutate(sample_id = x)) %>%
  rbindlist()  %>%
  mutate(mhc.a = str_split_fixed(mhc.a, "[:,]", 2)[,1]) %>%
  group_by(cdr3aa, antigen.epitope, antigen.species,
           mhc.a, sample_id, vdjdb.score, reference.id) %>%
  summarise(freq = sum(freq), count = sum(count)) %>%
  ungroup -> df.vdjdb

df.vdjdb %>%
  head
```

```
## # A tibble: 6 x 9
##   cdr3aa antigen.epitope antigen.species mhc.a sample_id vdjdb.score
##   <chr>  <chr>           <chr>           <chr> <chr>           <int>
## 1 CAAAG~ GILGFVFTL       InfluenzaA      HLA-~ control             0
## 2 CAAGG~ FLYNLLTRV       HomoSapiens     HLA-~ B35+                0
## 3 CAAGG~ FLYNLLTRV       HomoSapiens     HLA-~ control             0
## 4 CAAGG~ ELAGIGILTV      HomoSapiens     HLA-~ control             0
## 5 CAAGL~ LLWNGPMAV       YellowFeverVir~ HLA-~ control             1
## 6 CAAGR~ MLNIPSINV       CMV             HLA-~ control             0
## # ... with 3 more variables: reference.id <chr>, freq <dbl>, count <int>
```

```
nrow(df.vdjdb)
```

```
## [1] 115127
```

```
# Select unambigous assignments
df.vdjdb.good <- df.vdjdb %>%
  select(cdr3aa, antigen.epitope, mhc.a, vdjdb.score, reference.id) %>%
  unique %>%
  group_by(cdr3aa) %>%
  mutate(vdjdb.score.max = max(vdjdb.score)) %>%
  filter(vdjdb.score == vdjdb.score.max) %>%
  # In case of ties select the one with max # publications
  group_by(cdr3aa) %>%
  mutate(num.pub = str_count(reference.id, ","),
         num.pub.max = max(num.pub)) %>%
  filter(num.pub == num.pub.max) %>%
  # Remove all remaining ambigous cases
  group_by(cdr3aa) %>%
  mutate(n.epitopes = length(unique(antigen.epitope))) %>%
  filter(n.epitopes == 1) %>%
  ungroup
```

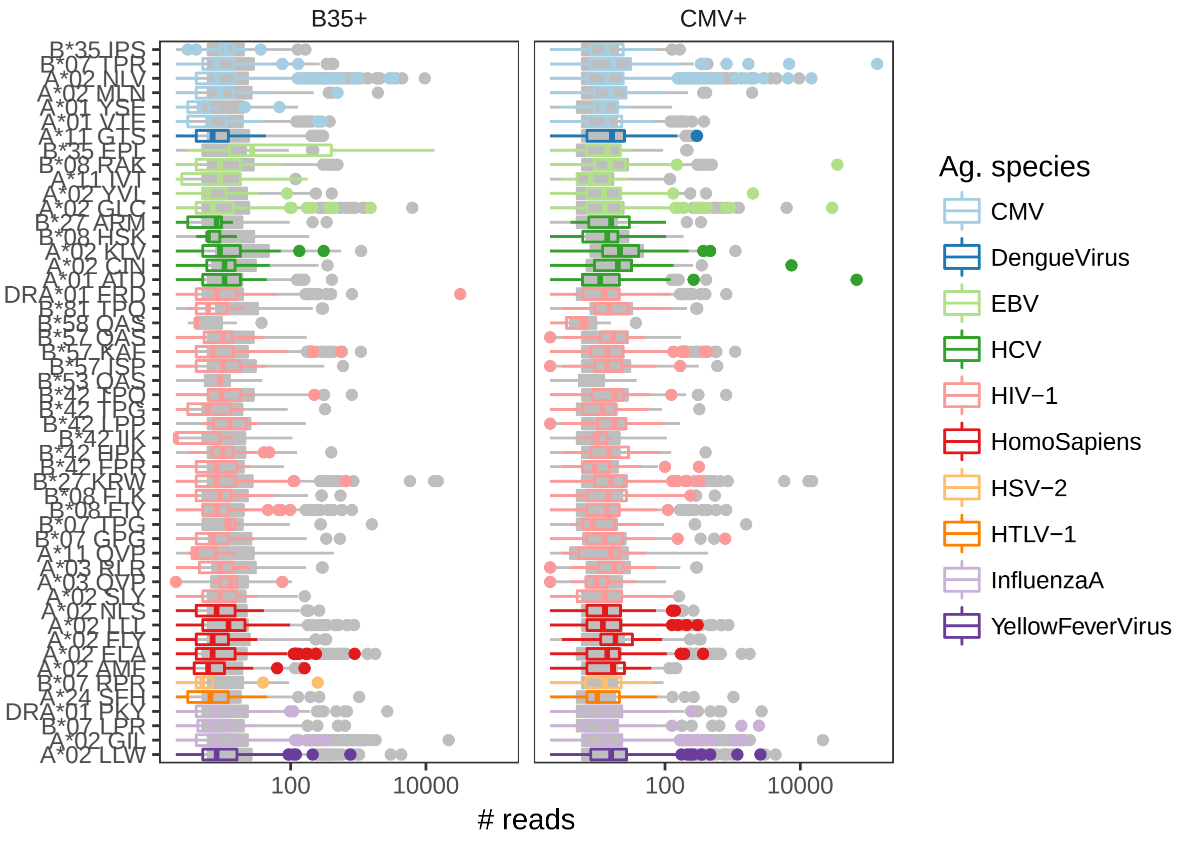
```r
# Apply filter
df.vdjdb <- df.vdjdb %>%
  merge(df.vdjdb.good)

# Some naming fixes
df.vdjdb <- df.vdjdb %>%
  mutate(epi.name = paste(substr(str_split_fixed(mhc.a, "[,:]", 2)[,1], 5, 10),
                          substr(antigen.epitope, 1, 3)),
         antigen.species = ifelse(startsWith(antigen.species,"DENV"),
                                  "DengueVirus",
                                  antigen.species))

nrow(df.vdjdb)
```

```
## [1] 66736
```

```r
# Split control
df.vdjdb.control <- df.vdjdb %>%
  filter(sample_id == "control")
df.vdjdb <- df.vdjdb %>%
  filter(sample_id != "control")
```

Plot all VDJdb annotations

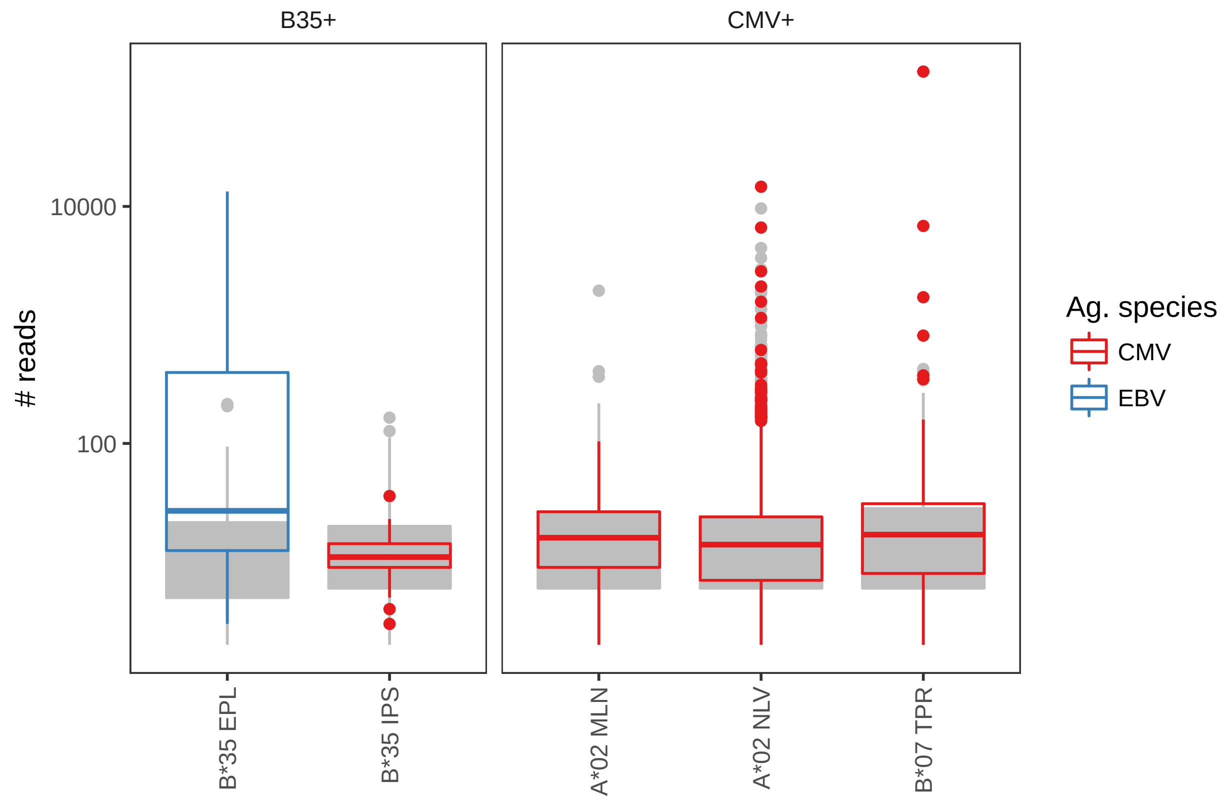
```r
df.vdjdb %>%
  ggplot(aes(x = fct_reorder2(epi.name,
                              freq,
                              as.integer(as.factor(antigen.species))),
             y = count,
             color = antigen.species)) +
  geom_boxplot(data = df.vdjdb.control %>% select(-sample_id),
               color = "grey", fill = "grey") +
  geom_boxplot(fill = NA) +
  coord_flip() +
  scale_y_log10("# reads") + xlab("") +
  scale_color_brewer("Ag. species", palette = "Paired") +
  facet_wrap(~sample_id) +
  theme_bw() +
  theme(panel.grid = element_blank(),
        strip.background = element_blank())
```



Zoom in/filter results based on donor HLA haplotype knowledge.

```r
df.vdjdb.f <- df.vdjdb %>%
  filter(
    (sample_id == "B35+" & startsWith(mhc.a, "HLA-A*26")) |
    (sample_id == "B35+" & startsWith(mhc.a, "HLA-A*33")) |
    (sample_id == "B35+" & startsWith(mhc.a, "HLA-B*14")) |
    (sample_id == "B35+" & startsWith(mhc.a, "HLA-B*35")) |
    (sample_id == "CMV+" & startsWith(mhc.a, "HLA-A*02") & antigen.species == "CMV") |
    (sample_id == "CMV+" & startsWith(mhc.a, "HLA-B*07") & antigen.species == "CMV") |
    (sample_id == "CMV+" & startsWith(mhc.a, "HLA-B*44") & antigen.species == "CMV")
    )

df.vdjdb.c <- df.vdjdb.control %>%
  mutate(sample_id = "B35+") %>%
  filter(startsWith(mhc.a, "HLA-A*26") |
         startsWith(mhc.a, "HLA-A*33") |
         startsWith(mhc.a, "HLA-B*14") |
         startsWith(mhc.a, "HLA-B*35") ) %>%
  rbind(
    df.vdjdb.control %>%
      mutate(sample_id = "CMV+") %>%
      filter(startsWith(mhc.a, "HLA-A*02") & antigen.species == "CMV" |
             startsWith(mhc.a, "HLA-B*07") & antigen.species == "CMV" |
             startsWith(mhc.a, "HLA-B*44") & antigen.species == "CMV"
             )
  )
```

```
df.vdjdb.f %>%
  ggplot(aes(x = fct_reorder2(epi.name,
                              freq,
                              as.integer(as.factor(antigen.species))),
             y = count,
             color = antigen.species)) +
  geom_boxplot(data = df.vdjdb.c,
               color = "grey", fill = "grey") +
  geom_boxplot(fill = NA) +
  scale_y_log10("# reads") + xlab("") +
  scale_color_brewer("Ag. species", palette = "Set1") +
  facet_grid(.~sample_id, scales = "free", space = "free") +
  theme_bw() +
  theme(panel.grid = element_blank(),
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1),
        strip.background = element_blank())
```



Searching for "expanded" TCR groups

We will not look at the actual number of reads per clonotype here, but do it the other way. We will search for groups of homologous TCR sequences that are unlikely to be found in the sample simply by chance. Here we run TCR neighbourhood enrichment test (TCRNET) to select TCR groups enriched in the memory compartment.

- `CalcDegreeStats` runs TCRNET routine
- `-o 1,0,1` sets the search scope - match with one substitution

- `-g2 vj` compute the number of clonotypes with the same V/J combination, corrects for differential V/J usage
- `-b data/control.txt.gz` specifies the control (background dataset)

```
run_java("vdjtools",
         "CalcDegreeStats -o 1,0,1 -g2 vj -b data/control.txt.gz data/CMV+.txt.gz data/B35+.txt.gz outpu
         T)
```

Let's have a look at TCRNET P-values, correct them and select enriched clonotypes

```
# Load all data
list("CMV+", "B35+") %>%
  lapply(function(x)
    "output/tcrnet.{x}.txt" %>%
      str_glue() %>%
      fread() %>%
      mutate(sample_id = x)) %>%
  rbindlist(fill = T) -> df.tcrnet

# Have a glance on output table
df.tcrnet %>%
  head
```

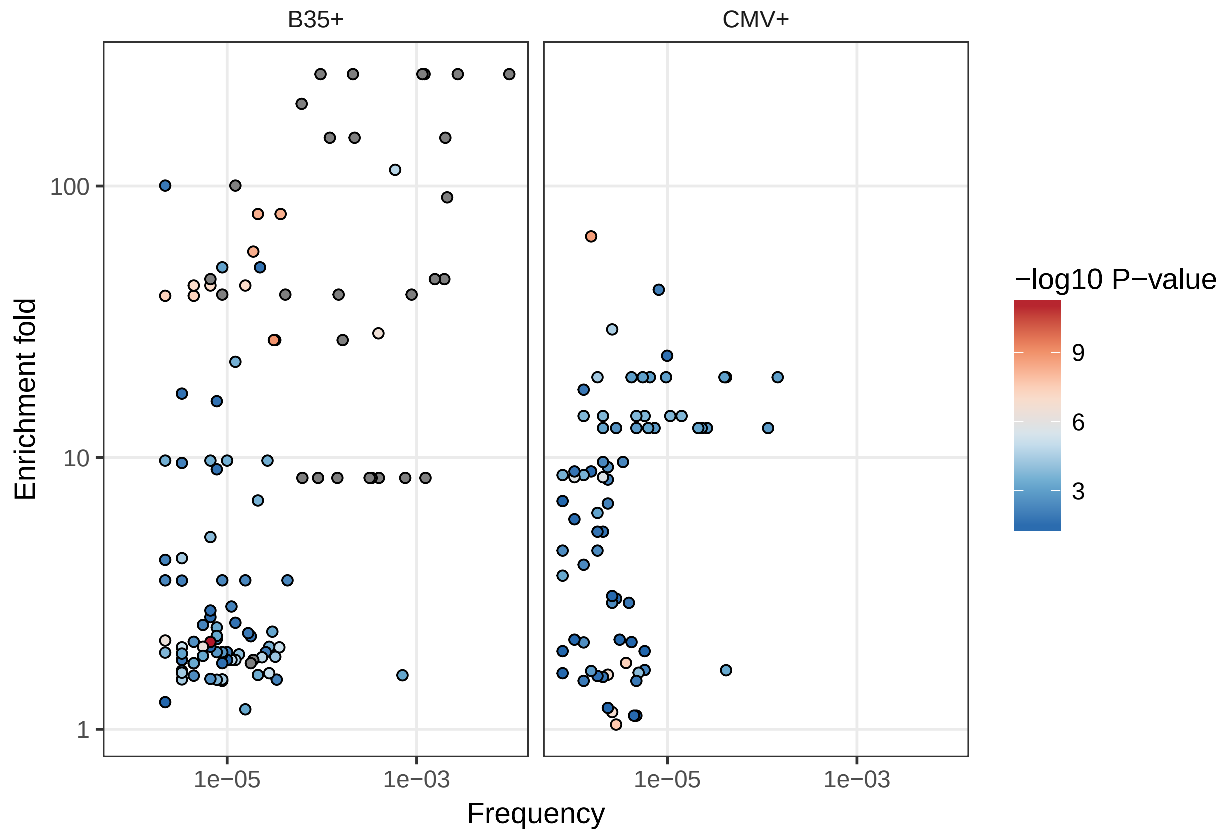
```
##     count       freq
## 1: 256397 0.06712129
## 2: 137460 0.03598518
## 3:  66664 0.01745174
## 4:  63072 0.01651140
## 5:  57317 0.01500482
## 6:  45167 0.01182411
##                                                         cdr3nt
## 1: TGCGCCAGCAGCCAAGATTGGGGGACAGACTCCCTATTCTCTGGAAACACCATATATTTT
## 2:               TGTGCCAGCAGCCTCCAGACAGGGTTGAACACTGAAGCTTTCTTT
## 3:                  TGTGCCAGCAGCTTAGTGGGGGGCGCGGGGGAGCAGTACTTC
## 4:                      TGTGCCAGCCCCTGAGCTAAATTAGAGAGCAGTACTTC
## 5:            TGTGCCAGCAGTTTATCGATTCGCAGGGCGGGCACTGAAGCTTTCTTT
## 6:               TGTGCCAGCAGTTTAGAAATCGCCGTGAACACTGAAGCTTTCTTT
##                 cdr3aa       v     d       j VEnd DStart DEnd JStart
## 1: CASSQDWGTDSLFSGNTIYF TRBV4-3 TRBD1 TRBJ1-3   18     21   28     38
## 2:      CASSLQTGLNTEAFF TRBV7-9 TRBD1 TRBJ1-1   12     17   24     25
## 3:       CASSLVGGAGEQYF TRBV7-9 TRBD1 TRBJ2-7   16     18   26     30
## 4:        CASP*A_IREQYF TRBV6-4 TRBD1 TRBJ2-7    9      9   14     26
## 5:     CASSLSIRRAGTEAFF  TRBV28 TRBD2 TRBJ1-1   16     23   28     32
## 6:      CASSLEIAVNTEAFF  TRBV28     . TRBJ1-1   15    -37  -37     25
##    degree.s group.count.s group2.count.s degree.c group.count.c
## 1:        1        154126             43        0        913905
## 2:        2        154126            850       26        913905
## 3:        4        154126           1422       12        913905
## 4:       -1            -1             -1       -1            -1
## 5:        1        154126            445        0        913905
## 6:        1        154126            445        2        913905
##    group2.count.c p.value.g p.value.g2 sample_id
## 1:            201 1.0000000  1.0000000      CMV+
## 2:           5609 0.9515390  0.9306782      CMV+
## 3:           7264 0.2016678  0.2738291      CMV+
## 4:             -1 1.0000000  1.0000000      CMV+
## 5:           5895 1.0000000  1.0000000      CMV+
## 6:           5895 1.0000000  1.0000000      CMV+
```

```
# Remove singletons, correct P-values
df.tcrnet <- df.tcrnet %>%
  group_by(sample_id) %>%
  mutate(p.adj = p.adjust(p.value.g2),
         fold = (degree.s + 1) / group.count.s /
           (degree.c + 1) * group.count.c) %>%
  ungroup

# Select enriched variants
df.tcrnet.e <- df.tcrnet %>%
  filter(p.adj < 0.05)

df.tcrnet.e %>%
  group_by(sample_id) %>%
  summarise(count = n())
```

```
## # A tibble: 2 x 2
##   sample_id count
##   <chr>     <int>
## 1 B35+        114
## 2 CMV+         74
```

Not much correlation between enrichment fold and clonotype frequency

```
# Volcano-like plot
df.tcrnet.e %>%
  ggplot(aes(x = freq, y = fold, fill = -log10(p.adj))) +
  geom_point(shape = 21) +
  scale_x_log10("Frequency") +
  scale_y_log10("Enrichment fold") +
  scale_fill_distiller("-log10 P-value", palette = "RdBu") +
  facet_wrap(~sample_id) +
  theme_bw() +
  theme(panel.grid.minor = element_blank(),
        strip.background = element_blank())
```



```r
df.tcrnet.e %>%
  group_by(sample_id) %>%
  do(cor.test(.$fold, .$freq, method = "spearman") %>% tidy)
```

```
## Warning in cor.test.default(.$fold, .$freq, method = "spearman"): Cannot
## compute exact p-value with ties

## Warning in cor.test.default(.$fold, .$freq, method = "spearman"): Cannot
## compute exact p-value with ties

## # A tibble: 2 x 6
## # Groups:   sample_id [2]
##   sample_id estimate statistic   p.value method                alternative
##   <chr>        <dbl>     <dbl>     <dbl> <fct>                 <fct>
## 1 B35+         0.419   143457.   3.49e-6 Spearman's rank corr~ two.sided
## 2 CMV+         0.305    46915.   8.18e-3 Spearman's rank corr~ two.sided
```

Extracting enriched groups of homologous TCRs

Compute graph with 1 substitution allowed. Here we'll use all clonotypes (except singletons) that are neighbours of enriched clonotypes.

```r
# Hamming distance
find_pairs <- function(x, y) {
  res <- stringdistmatrix(x, y,
                          method = "hamming",
                          useNames = "strings",
                          nthread = CORES) %>%
    melt %>%
    filter(value == 1) %>%
    select(-value)
  colnames(res) <- c("from.cdr3", "to.cdr3")
  res
}

# Graph data frame
df.tcrnet.e %>%
  .$sample_id %>%
  unique %>%
  as.list %>%
  lapply(function(x)
    find_pairs(df.tcrnet.e %>% filter(sample_id == x) %>% .$cdr3aa %>% unique,
               df.tcrnet %>% filter(sample_id == x) %>% .$cdr3aa %>% unique) %>%
      mutate(sample_id = x)
    ) %>%
  rbindlist -> df.graph

df.graph %>%
  head
```

```
##             from.cdr3           to.cdr3 sample_id
## 1:     CASSLQGYEQYF      CASSLAGYEQYF      CMV+
## 2: CASSLLGQASSYEQYF CASSLEGQASSYEQYF      CMV+
## 3: CASSLEGQASTYEQYF CASSLEGQASSYEQYF      CMV+
## 4:    CASSYSPGGTQYF    CASSQSPGGTQYF      CMV+
## 5:    CASSQSPGGTQYF    CASSQSPGGIQYF      CMV+
## 6:    CASSLGPSYEQYF    CASSLGQSYEQYF      CMV+
```

Layout and plot graphs. Highlight connected components/clusters

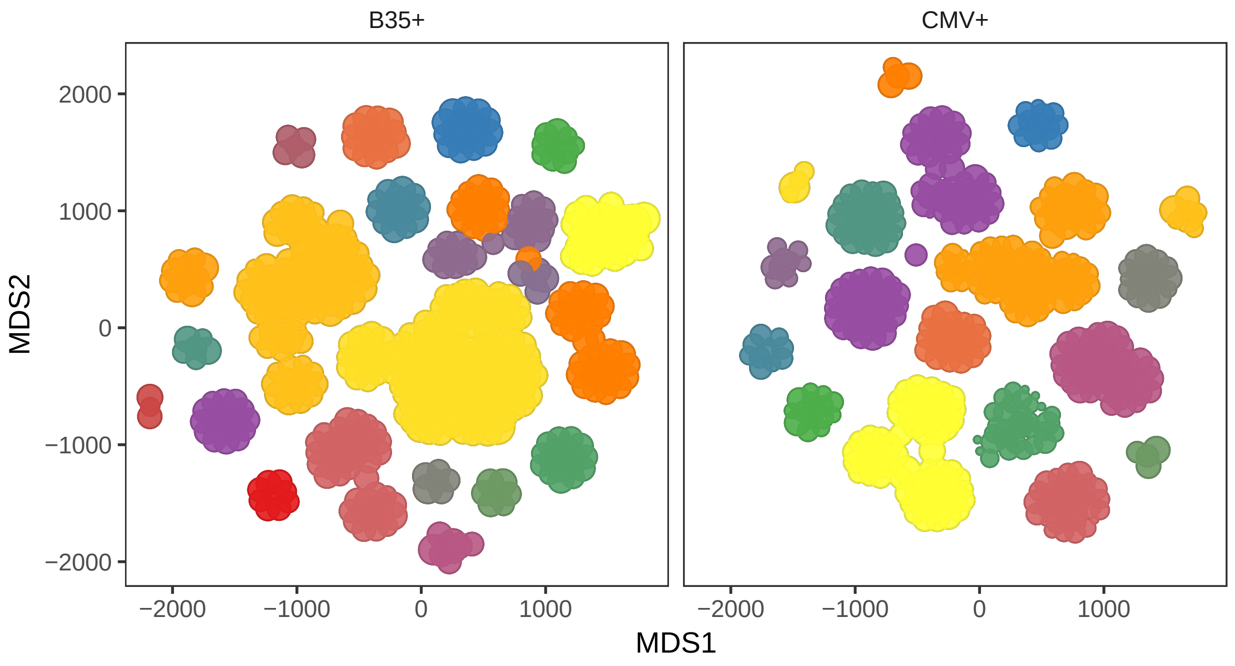
```
# graph layout/component naming function
layout_graph <- function(graph) {
  set.seed(42)

  gg <- graph %>%
    select(-sample_id) %>%
    graph_from_data_frame %>%
    simplify

  cc <- clusters(gg)

  coords <- gg %>%
      layout_with_graphopt(niter = 3000, charge = 0.005)

  data.frame(cdr3aa = names(V(gg)),
             x = coords[,1],
             y = coords[,2],
             stringsAsFactors = F) %>%
    merge(
      data.frame(cdr3aa = names(cc$membership),
                 cid = cc$membership,
                 cid2 = paste0(graph$sample_id[1], "_C", cc$membership)))
}
```

```
# apply to both samples
df.mds <- df.graph %>%
  group_by(sample_id) %>%
  do(layout_graph(.)) %>%
  ungroup %>%
  merge(df.tcrnet %>%
          group_by(cdr3aa, sample_id) %>%
          summarise(freq = sum(freq)),
        by = c("cdr3aa", "sample_id"))
```

```
## Warning in bind_rows_(x, .id): Unequal factor levels: coercing to character
```

```
## Warning in bind_rows_(x, .id): binding character and factor vector,
## coercing into character vector

## Warning in bind_rows_(x, .id): binding character and factor vector,
## coercing into character vector
```

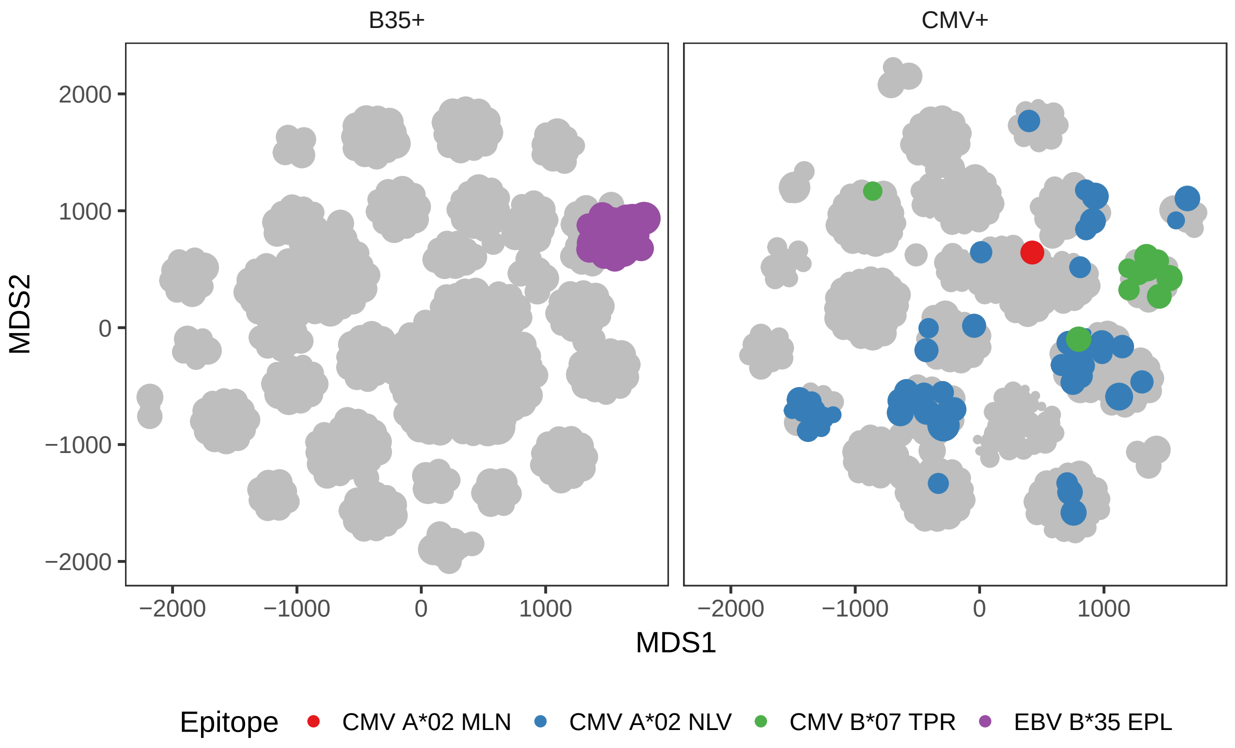
```
# plot 2D graph layout colored by connected component
df.mds %>%
  ggplot(aes(x = x, y = y,
             size = log10(freq))) +
  geom_point(shape = 21) +
  geom_point(aes(color = as.integer(factor(cid))), alpha = 0.9) +
  xlab("MDS1") + ylab("MDS2") +
  scale_color_distiller(guide = F, palette = "Set1") +
  scale_size(guide = F) +
  facet_wrap(~sample_id) +
  theme_bw() +
  theme(aspect = 1,
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank())
```



Combining TCRNET results and VDJdb annotations

Color graph by annotations

```
# append annotations
df.mds.ag.freq <- df.mds %>%
  merge(df.vdjdb.f %>%
          mutate(epi.name = paste(antigen.species, epi.name)) %>%
          select(cdr3aa, epi.name, sample_id) %>% unique,
        all.x = T, by = c("cdr3aa", "sample_id"))

# plot graph layout colored by annotation
ggplot(df.mds.ag.freq %>% filter(!is.na(epi.name)),
       aes(x = x, y = y, color = factor(epi.name),
           size = log10(freq)
           )) +
  geom_point(data = df.mds.ag.freq, color = "grey") +
  geom_point() +
  xlab("MDS1") + ylab("MDS2") +
  scale_color_brewer("Epitope", palette = "Set1") +
  scale_size(guide = F) +
  facet_wrap(~sample_id) +
  theme_bw() +
  theme(aspect = 1,
        legend.position = "bottom",
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank(),
        strip.background = element_blank())
```



Get IDs of interesting clusters

```r
df.mds.ag.freq %>%
  group_by(cid2) %>%
  mutate(total = n()) %>%
  filter(!is.na(epi.name)) %>%
  group_by(cid2, epi.name) %>%
  # fraction annotated of all nodes in component
  summarise(fraction.annot = n() / total[1]) %>%
  arrange(-fraction.annot)
```

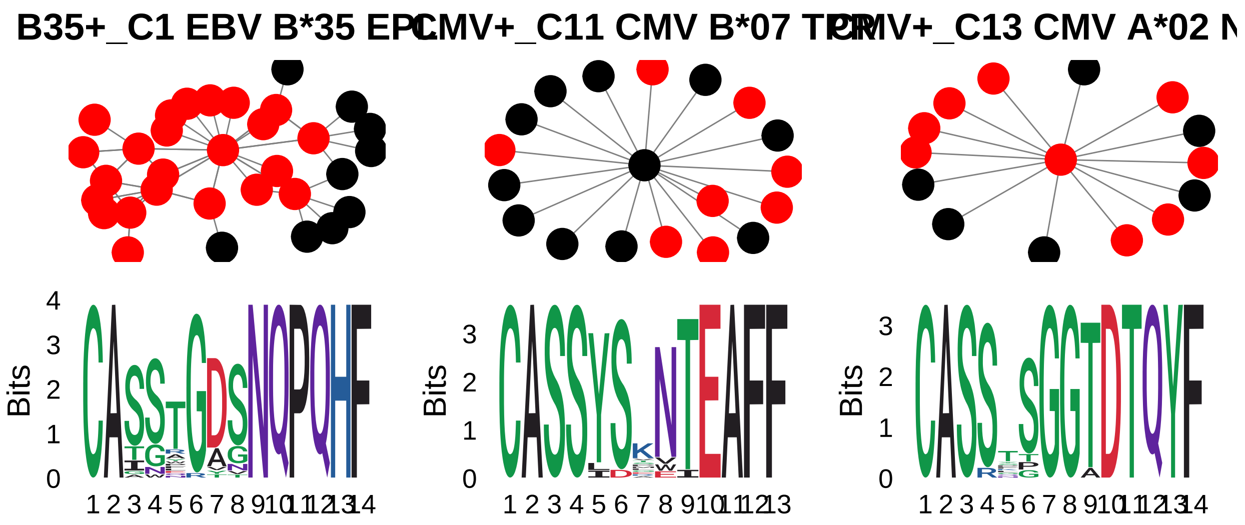
```
## # A tibble: 13 x 3
## # Groups:   cid2 [11]
##    cid2     epi.name     fraction.annot
##    <chr>    <chr>                 <dbl>
##  1 B35+_C1  EBV B*35 EPL        0.719
##  2 CMV+_C13 CMV A*02 NLV        0.6
##  3 CMV+_C11 CMV B*07 TPR        0.421
##  4 CMV+_C3  CMV A*02 NLV        0.286
##  5 CMV+_C8  CMV A*02 NLV        0.268
##  6 CMV+_C1  CMV A*02 NLV        0.15
##  7 CMV+_C6  CMV A*02 NLV        0.107
##  8 CMV+_C17 CMV A*02 NLV        0.0769
##  9 CMV+_C7  CMV A*02 NLV        0.0769
## 10 CMV+_C4  CMV A*02 NLV        0.0594
## 11 CMV+_C15 CMV B*07 TPR        0.0303
## 12 CMV+_C8  CMV B*07 TPR        0.0179
## 13 CMV+_C4  CMV A*02 MLN        0.00990
```

Plotting motifs

```r
# fetching sequences
get_seqs_cid <- function(cc) {
  df.mds.ag.freq %>%
    filter(cid2 == cc) %>%
    .$cdr3aa
}

# multiple sequence alignment
align_seqs <- function(seqs, cons = F) {
  x <- seqs %>% AAStringSet %>% msa(method = "ClustalW")

  if (cons) {
    return(msaConsensusSequence(.x))
  } else {
    return(x %>%
          as.matrix %>%
          melt %>%
          mutate(seq_id = Var1, base_id = Var2, aa = value) %>%
          select(-Var1, -Var2, -value) %>%
          group_by(seq_id) %>%
          mutate(seq = paste0(aa[base_id], collapse = "")) %>%
          ungroup)
  }
}

## Plotting
```

```r
# plots a grid of AAs from multiple alignment
plot_seqgrid <- function(seqs) {
  seqs %>%
    align_seqs %>%
    ggplot(aes(x=base_id, y=seq_id)) +
    geom_text(aes(label=aa), size = 3) +
    scale_x_continuous("", breaks = c(),
                       expand = c(0.105, 0)) +
    theme_logo() +
    theme(legend.position = 'none')
}

# plots sequence logo from multiple alignment
plot_seqlogo <- function(seqs) {
  seqs %>% align_seqs %>% .$seq %>% unique %>% ggseqlogo +
    theme(legend.position = 'none')
}

# plots graph using igraph
plot_seqgraph <- function(cc, epitope) {
  set.seed(42)
  ss <- (df.mds.ag.freq %>%
    filter(cid2 == cc) %>%
    .$sample_id)[1]

  seqs <- get_seqs_cid(cc)

  df.graph %>%
    filter(sample_id == ss, to.cdr3 %in% seqs | from.cdr3 %in% seqs) %>%
    select(to.cdr3, from.cdr3) %>%
    unique %>%
    as.matrix %>%
    network -> nn

  seqs_annot <- df.mds.ag.freq %>%
    filter(epi.name == epitope & cid2 == cc) %>%
    .$cdr3aa

  grp <- ifelse(network.vertex.names(nn) %in% seqs_annot, "g1", "g2")
  nn %v% "group" <- grp
  clrs <- c("black", "red")
  names(clrs) <- c("g2", "g1")

  nn %>% ggnet2(color = "group",
              size = 5,
              color.palette = clrs,
              legend.position = "none") +
    ggtitle(paste(cc, epitope))
}

# make all plots
plot_cid_full <- function(cc) {
  plotlist <- cc %>% strsplit(",") %>% lapply(function(x)
    plot_seqgraph(x[1], x[2])
    )
  #plotlist <- c(plotlist,
  #               cc %>% as.list %>% lapply(function(x)
  #                 x %>% get_seqs_cid %>% plot_seqgrid
  #                 )
  #               )
  plotlist <- c(plotlist,
                cc %>% strsplit(",") %>% lapply(function(x)
                  x[1] %>% get_seqs_cid %>% plot_seqlogo
                  )
                )

  plotlist
}

plot_grid(plotlist = plot_cid_full(c("B35+_C1,EBV B*35 EPL",
                                     "CMV+_C11,CMV B*07 TPR",
                                     "CMV+_C13,CMV A*02 NLV")),
          ncol = 3, nrow = 3, align = 'v')
```

```
## use default substitution matrix
## use default substitution matrix
## use default substitution matrix
```



Something we have missed

```
get_top_clonotypes <- function(allele) {
  df.vdjdb.f %>%
  filter(sample_id == "CMV+") %>%
  filter(startsWith(mhc.a, allele)) %>%
  select(count, freq, cdr3aa) %>%
  arrange(-count) %>%
  head(10)
}

get_top_clonotypes("HLA-B*07")
```

```
##     count         freq           cdr3aa
## 1  137472 3.598832e-02  CASSLQTGLNTEAFF
## 2    6846 1.792191e-03    CASSPSRNTEAFF
## 3    1713 4.484404e-04    CASSPHRNTEAFF
## 4     813 2.128325e-04 CASSFRQGIDTGELFF
## 5     375 9.816995e-05     CASSYSSGELFF
## 6     348 9.110172e-05     CASSYSHGELFF
## 7     159 4.162406e-05 CASSLRDGINTGELFF
## 8     154 4.031513e-05 CASSLRQGANTGELFF
## 9     143 3.743548e-05    CASSYSRNTEAFF
## 10    143 3.743548e-05    CASSYSRNTEAFF
```

```
get_top_clonotypes("HLA-A*02")
```

```
##    count         freq           cdr3aa
## 1  14664 0.0038388379     CASSLGQDTQYF
## 2   6633 0.0017364302      CASSSVNEQFF
## 3   2834 0.0007419031     CASLQGNTEAFF
## 4   2110 0.0005523696      CASSSVGGYTF
## 5   1570 0.0004110049     CASSLAGYEQYF
## 6   1146 0.0003000074    CASSPTGNYGYTF
## 7    615 0.0001609987    CASSQEGSQPQHF
## 8    473 0.0001238250   CASSYSADTGELFF
## 9    472 0.0001235632  CASSLDILSYNEQFF
## 10   463 0.0001212072 CASSLAPGATNEKLFF
```