Creation of active gene-lists

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Contents

1 Active/Inactive Gene lists

Our aim is to create a unified table that assigns to each gene in the P.falicparum gnome a expresion state. We will define 5 possible expresion states:

- Active
  - Active (no-variant genes)
  - Variant Active
  - Variant Repressed
- Inactive (no-variant genes)
- Not-Settable

1.1 Microarray Data: Red Signal

We willl load the red signal and transform it into percentiles. For each gene we pick the "Aver.2Higher" column from the original microarrays data table. This column corresponds to the average between the two highest red signals among available timepoints.

Red Signal DataFrame

```
        Gene_id     Red_12B     Red_10G   Red_3D7B Percent_12B Percent_10G
1    mal_mito_3 22579.33333 36436.73333 30636.8250  96.0466005  98.4721161
2 PF3D7_0100100   104.17083   215.03542   264.4000   5.6913675  10.9625668
3 PF3D7_0100200  1357.70000    72.67917   724.1500  29.7555386   4.0106952
4 PF3D7_0100300   283.98333   291.69583  7540.9042  12.5668449  12.9870130
5 PF3D7_0100400   193.77500    35.01250   161.0833   9.8930481   1.0504202
6 PF3D7_0100600    31.58333    33.40417    31.5250   0.2291826   0.4392666
  Percent_3D7B MaxRedPercentDif MeanRedPercent
1   97.8800611         2.425516     97.4662592
2   11.1153552         5.423988      9.2564298
3   19.9006875        25.744843     17.8889738
4   81.3407181        68.773873     35.6315253
5    8.0595875         8.842628      6.3343519
6    0.2387319         0.210084      0.3023937
```

1.2 Microarray Data: Areas

We will load the areas data to calculate FC among strains. For each gene, we select the time interval (right, left, mid or sides) for which we find the

maximum difference among strains (between highest and lowest). We will also add a column to check if this time interval corresponds to the interval of maxium expression for each strain.

Areas DataFrame

	Gene_id	l_12B	r_12B	m_12B	s_12B	l_10G	r_10G
1	mal_mito_3	30.592496	61.080128	49.676556	41.99607	25.372470	62.38873
2	MAL13P1.415_oldname	5.423269	8.488971	1.289779	12.62246	6.117132	10.59524
3	MAL13P1.65_oldname	18.322430	NA	17.593468	NA	14.071128	NA
4	MAL7P1.142_oldname	9.389247	12.807814	10.340803	11.85626	13.661078	14.52676
5	MAL8P1.310_oldname	NA	NA	NA	NA	NA	NA
6	MAL8P1.90_oldname	NA	NA	NA	NA	NA	NA

	m_10G	s_10G	l_3D7B	r_3D7B	m_3D7B	s_3D7B	MaxLeft	MinLeft
1	49.805504	37.95570	25.484634	62.83441	50.462696	37.856349	30.592496	25.372470
2	3.676218	13.03616	1.789873	10.51234	3.691753	8.610459	6.117132	1.789873
3	NA	NA	19.333324	NA	NA	NA	19.333324	14.071128
4	13.401610	14.78623	7.099032	13.34518	12.041177	8.403034	13.661078	7.099032
5	NA	NA	NA	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA	NA	NA	NA

	MaxRight	MinRight	MaxMid	MinMid	MaxSides	MinSides	DifLeft	DifRight
1	62.83441	61.080128	50.462696	49.676556	41.99607	37.856349	5.220025	1.754283
2	10.59524	8.488971	3.691753	1.289779	13.03616	8.610459	4.327259	2.106274
3	NA	NA	NA	NA	NA	NA	5.262196	NA
4	14.52676	12.807814	13.401610	10.340803	14.78623	8.403034	6.562046	1.718950
5	NA	NA	NA	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA	NA	NA	NA

	DifMid	DifSides	Interval	MaxDif	areaFC	area_12B	area_10G	area_3D7B
1	0.7861401	4.139719	Left	5.220025	0.3881060	30.592496	25.37247	25.484634
2	2.4019733	4.425700	Sides	4.425700	0.3290483	12.622460	13.03616	8.610459
3	NA	NA	Left	5.262196	0.3912413	18.322430	14.07113	19.333324
4	3.0608067	6.383199	Left	6.562046	0.4878845	9.389247	13.66108	7.099032
5	NA	NA	No Data	NA	NA	NA	NA	NA
6	NA	NA	No Data	NA	NA	NA	NA	NA

	MaxArea	MinArea
1	30.59250	25.372470
2	13.03616	8.610459
3	19.33332	14.071128
4	13.66108	7.099032
5	NA	NA
6	NA	NA

1.3 Load RNA-Seq Data

We will use publicly available data from PlasmoDB to create a reference expresion percentile for each gene. All data-sets are from RNA-Seq studies in the 3D7 strain. We are using 4 different data-sets:

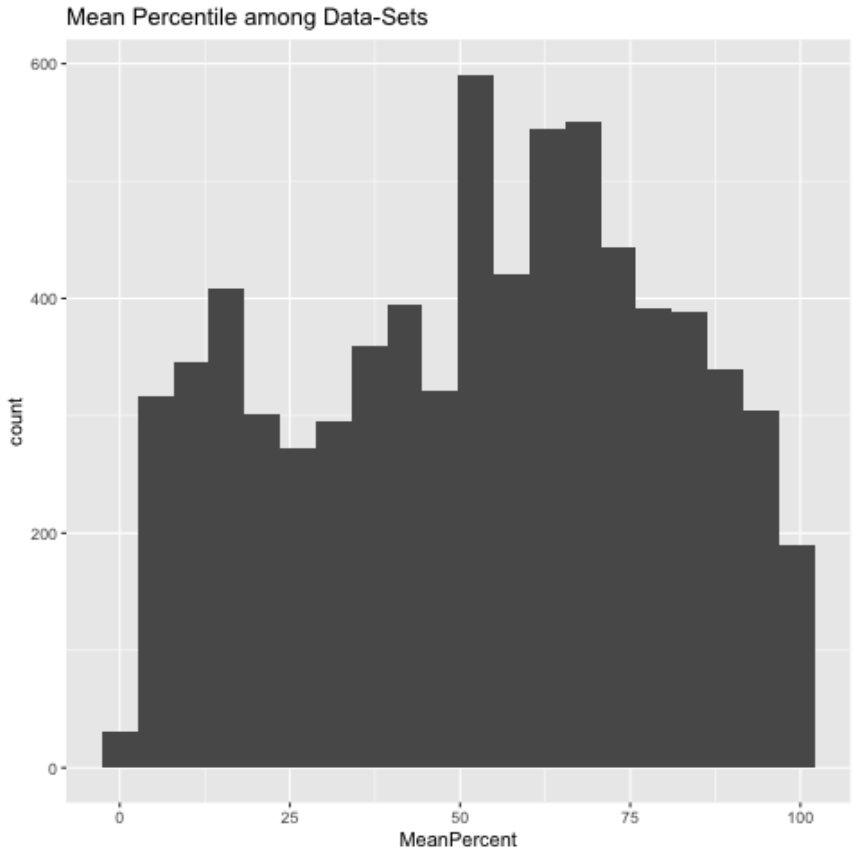
- Otto et.al.
- Hoeijmakers et.al.
- Toenhake et.al.
- Bartfai et.al.

We use only uniquely mapped reads and scaled data when available. For each Data-Set we first create a column representing the maxium value among timepoints for each gene. We then convert this column into percentile values. Finally we average this percentile values among all Data-Sets.

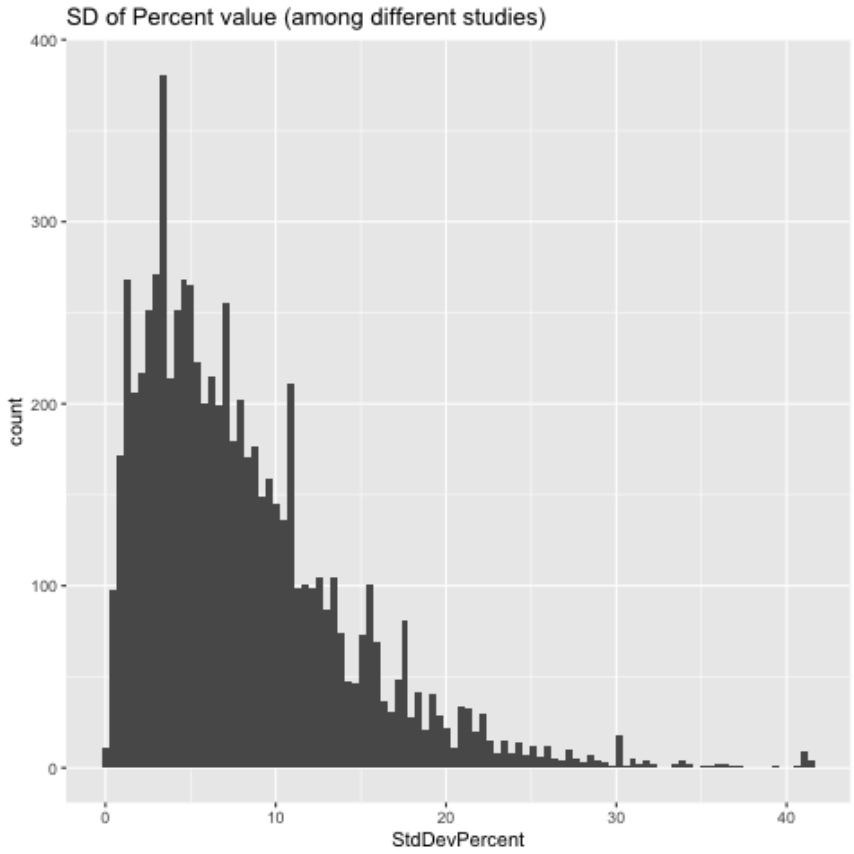
RNA-Seq DataFrame

```
        Gene_id Otto_Max_pcnt Hoeij_Max_pcnt Toen_Max_pcnt Bart_Max_pcnt
1 PF3D7_0100100     26.104032      24.855996     17.577239     18.240531
2 PF3D7_0100200      7.366032      25.693838     13.833130     18.764182
3 PF3D7_0100300     22.045732       3.587013      2.469890      1.885146
4 PF3D7_0100400     15.290627      17.315413     10.830861     14.993891
5 PF3D7_0100500     25.885844      16.145924     24.471985      9.399546
6 PF3D7_0100600      7.915867       4.468494      3.717926      3.054634
  MeanPercent StdDevPercent
1   21.694449      3.818402
2   16.414296      6.711285
3    7.496945      8.421970
4   14.607698      2.356468
5   18.975825      6.664638
6    4.789230      1.873182
```

We plot the resulting distribution of percentiles (it should be almost flat).



We plot the standard deviation of the percentile values among different Data-Sets and we can see that for the vast majority of genes it doesn't go above 10.



1.4 Create table for classification

Joining and summarizing all the prevoius data we create a a table that will let us classify the genes according to their expression state.

To classify genes, we add a colum for each strain were we classify genes according to their expression relative to other strains. To create this column we have taken the maximum and minimum area value for each gene and divided this interval in 3 equal regions. Each strain then gets a value of man/mid/max according to which region it falls.

`gene-min---|--mid---|---gene-max`

Final DataFrame

```
        Gene_id Variant Percent_12B Percent_10G Percent_3D7B MaxRedPercentDif
1    mal_mito_3   FALSE  96.0466005  98.4721161   97.8800611         2.425516
```

```
2 PF3D7_0100100    TRUE   5.6913675  10.9625668   11.1153552         5.423988
3 PF3D7_0100200    TRUE  29.7555386   4.0106952   19.9006875        25.744843
4 PF3D7_0100300    TRUE  12.5668449  12.9870130   81.3407181        68.773873
5 PF3D7_0100400    TRUE   9.8930481   1.0504202    8.0595875         8.842628
6 PF3D7_0100600    TRUE   0.2291826   0.4392666    0.2387319         0.210084
  MeanRedPercent Interval    areaFC area_12B  area_10G area_3D7B  MaxArea
1     97.4662592     Left 0.3881060 30.59250 25.372470  25.48463 30.59250
2      9.2564298     Left 0.8879945 31.76998 43.713503  38.29839 43.71350
3     17.8889738    Right 3.5015562 52.33320  5.237266  43.02460 52.33320
4     35.6315253     Left 3.3957657 31.78908 33.073957  77.46213 77.46213
5      6.3343519     Left 2.0671154 23.21846  7.706832  35.50954 35.50954
6      0.3023937  No Data        NA       NA        NA        NA       NA
    MinArea MeanPercent rel_12B rel_10G rel_3D7B Gene_name
1 25.372470   92.568511     max     min      min      null
2 31.769976   21.694449     min     max      mid       VAR
3  5.237266   16.414296     max     min      max       RIF
4 31.789080    7.496945     min     min      max       VAR
5  7.706832   14.607698     mid     min      max       RIF
6        NA    4.789230      NA      NA       NA       RIF
                                  Annot
1                   unspecified product
2 erythrocyte membrane protein 1, PfEMP1
3                                 rifin
4 erythrocyte membrane protein 1, PfEMP1
5                                 rifin
6                                 rifin
```

1.5 Create Lists according to thresholds

Now that we have all the data loaded in, we can star to set labels for each gene.

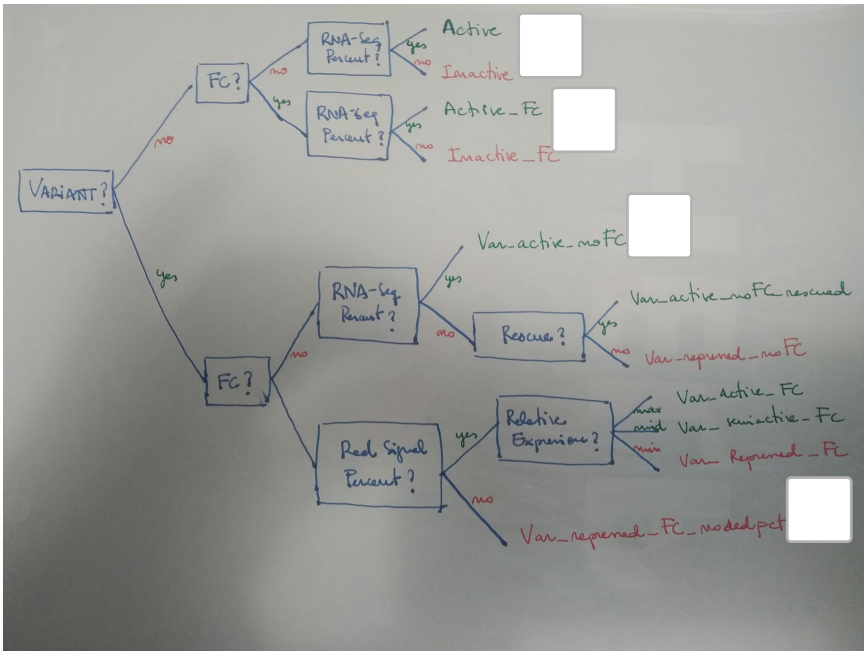
We have set the following thresholds:

- (rna_pcnt) RNA-Seq mean percentile: 25%
- (red_pcnt) Red Signal Percentile (by sample): 25%
- (red_rescue) Red Signal Mean Percentile for "rescuing": 40%
- (area_fc) Area log2 Fold-Change: 1

In addition to these thresholds we will use 2 more columns to set the categories:

- Variant: a column stating if gene is variant/non-variant
- Relative Expression (by strain): a colum were each gene is set to min/mid/max according to it's expression level relative to the other strains.

We will have the following categories with the following logic:



Genes that lack either an areaFC value or a RNA-Seq mean percentile are currently set to "Non-Settable".

Gene-State DataFrame

```
        Gene_id Variant Percent_12B Percent_10G Percent_3D7B MaxRedPercentDif
1    mal_mito_3   FALSE  96.0466005  98.4721161   97.8800611         2.425516
2 PF3D7_0100100    TRUE   5.6913675  10.9625668   11.1153552         5.423988
3 PF3D7_0100200    TRUE  29.7555386   4.0106952   19.9006875        25.744843
4 PF3D7_0100300    TRUE  12.5668449  12.9870130   81.3407181        68.773873
5 PF3D7_0100400    TRUE   9.8930481   1.0504202    8.0595875         8.842628
6 PF3D7_0100600    TRUE   0.2291826   0.4392666    0.2387319         0.210084
  MeanRedPercent Interval    areaFC area_12B  area_10G area_3D7B  MaxArea
1     97.4662592     Left 0.3881060 30.59250 25.372470  25.48463 30.59250
```

```
2      9.2564298     Left 0.8879945 31.76998 43.713503  38.29839 43.71350
3     17.8889738    Right 3.5015562 52.33320  5.237266  43.02460 52.33320
4     35.6315253     Left 3.3957657 31.78908 33.073957  77.46213 77.46213
5      6.3343519     Left 2.0671154 23.21846  7.706832  35.50954 35.50954
6      0.3023937  No Data        NA       NA        NA        NA       NA
    MinArea MeanPercent rel_12B rel_10G rel_3D7B Gene_name
1 25.372470   92.568511     max     min      min      null
2 31.769976   21.694449     min     max      mid       VAR
3  5.237266   16.414296     max     min      max       RIF
4 31.789080    7.496945     min     min      max       VAR
5  7.706832   14.607698     mid     min      max       RIF
6        NA    4.789230      NA      NA       NA       RIF
                                  Annot                   state_12B
1                   unspecified product                      Active
2 erythrocyte membrane protein 1, PfEMP1         Var_Repressed_noFC
3                                 rifin              Var_Active_FC
4 erythrocyte membrane protein 1, PfEMP1 Var_Repressed_FC_noredpcnt
5                                 rifin Var_Repressed_FC_noredpcnt
6                                 rifin               Not_settable
                  state_10G                 state_3D7B  category_12B
1                    Active                     Active        Active
2        Var_Repressed_noFC         Var_Repressed_noFC Var_Repressed
3 Var_Repressed_FC_noredpcnt Var_Repressed_FC_noredpcnt    Var_Active
4 Var_Repressed_FC_noredpcnt              Var_Active_FC Var_Repressed
5 Var_Repressed_FC_noredpcnt Var_Repressed_FC_noredpcnt Var_Repressed
6              Not_settable               Not_settable  Not_settable
   category_10G category_3D7B
1        Active        Active
2 Var_Repressed Var_Repressed
3 Var_Repressed Var_Repressed
4 Var_Repressed    Var_Active
5 Var_Repressed Var_Repressed
6  Not_settable  Not_settable
```

1.6 Some results

This is a table with the number of genes in each state for each strain.

States Table

```
  Strain Active Active_FC Inactive Inactive_FC Not_settable Var_Active_FC
```

```
1    12B   7025        29     1110          36          655             8
2    10G   7025        29     1110          36          655             7
3   3D7B   7025        29     1110          36          655            89
  Var_Active_noFC Var_Active_noFC_rescued Var_Repressed_FC
1             118                       3                7
2             118                       3                5
3             118                       3                3
  Var_Repressed_FC_noredpcnt Var_Repressed_noFC Var_Semiactive_FC
1                        168                128                 1
2                        172                128                NA
3                         92                128                NA
```

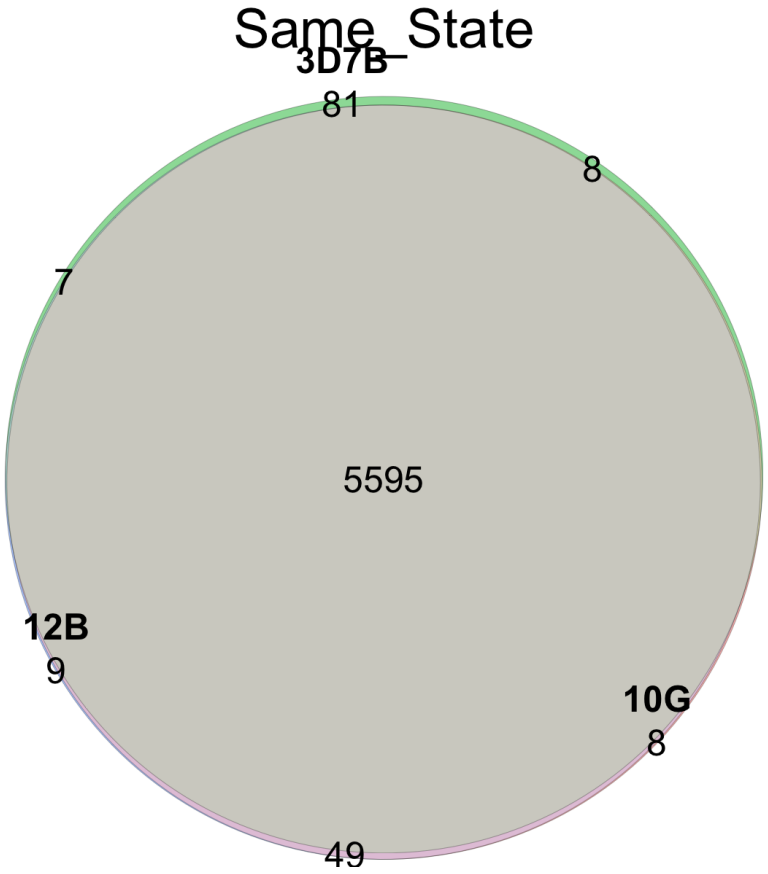
And this are the Clag genes

```
       Gene_id Variant Percent_12B Percent_10G Percent_3D7B MaxRedPercentDif
1 PF3D7_0302200    TRUE    78.68602    99.57983     42.32238         57.25745
2 PF3D7_0302500    TRUE    98.81589    84.39649     98.98778         14.59129
  MeanRedPercent Interval   areaFC  area_12B area_10G area_3D7B   MaxArea
1       73.52941    Right 4.823089  37.32948 88.19577  23.32522  88.19577
2       94.06672    Right 3.452270 100.12065 53.68762  99.58767 100.12065
   MinArea MeanPercent rel_12B rel_10G rel_3D7B Gene_name
1 23.32522    76.36804     min     max      min   CLAG3.2
2 53.68762    88.72404     max     min      max   CLAG3.1
                                    Annot        state_12B        state_10G
1 cytoadherence linked asexual protein 3.2 Var_Repressed_FC    Var_Active_FC
2 cytoadherence linked asexual protein 3.1    Var_Active_FC Var_Repressed_FC
       state_3D7B  category_12B  category_10G category_3D7B
1 Var_Repressed_FC Var_Repressed    Var_Active Var_Repressed
2    Var_Active_FC    Var_Active Var_Repressed    Var_Active
```

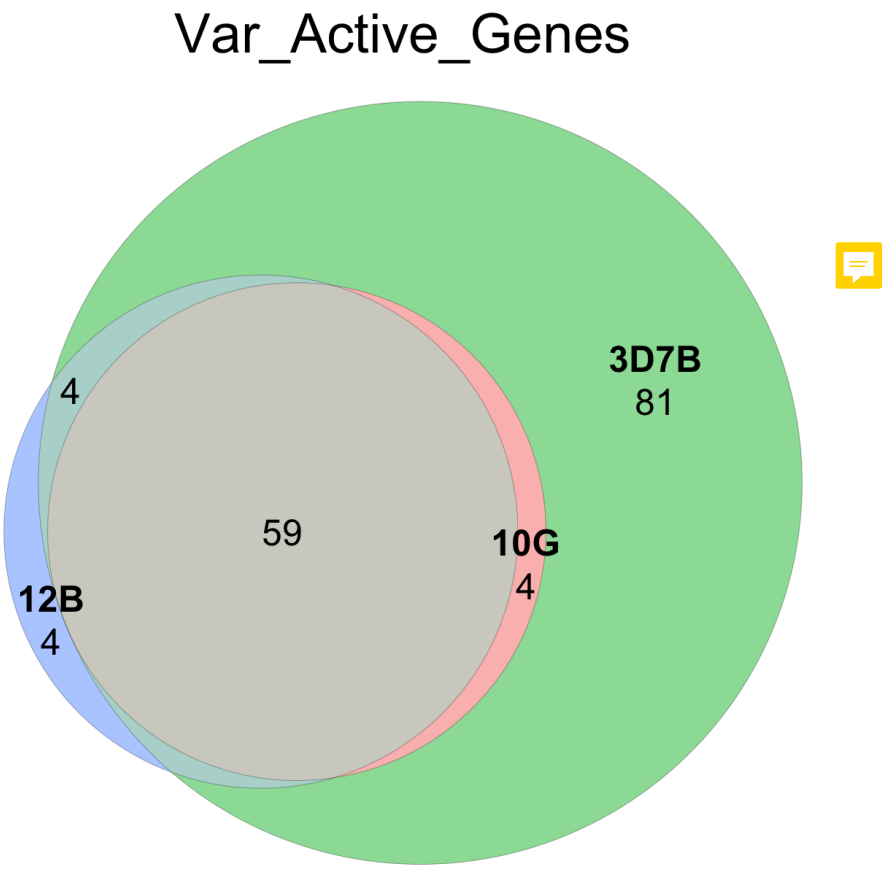
1.7 Venn Diagrams

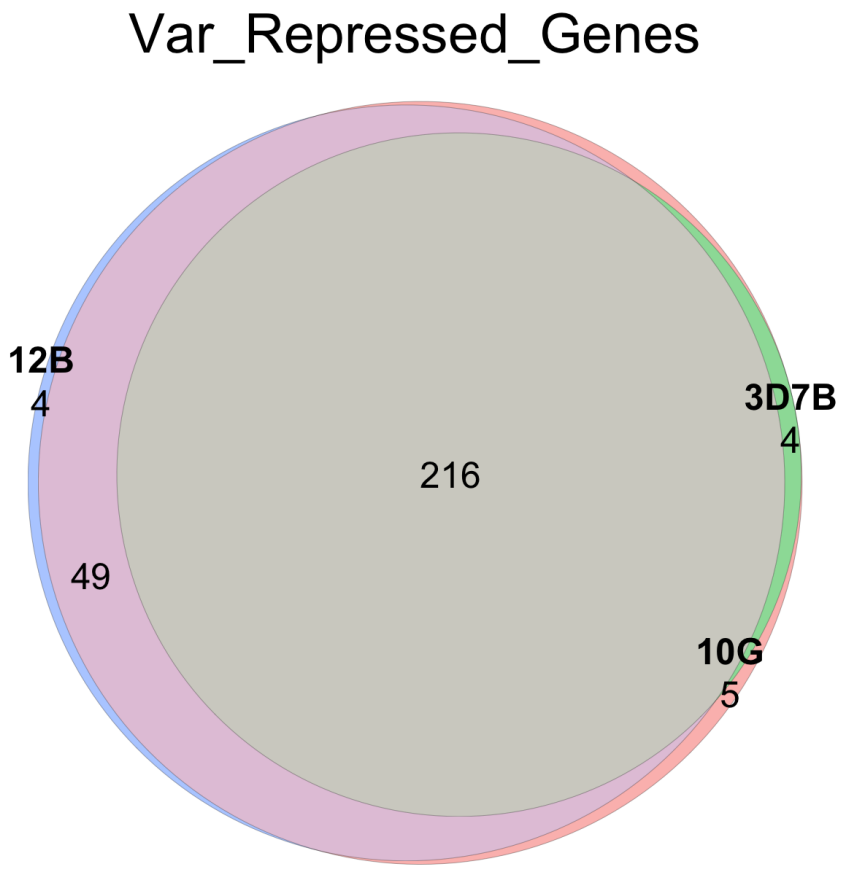
Here we plon Venn diagrams to visualize how the strains compare.

First we check how many genes share the same "state" among strains:



Then we make venn diagrams for the "Var_active" and "Var_Repressed" states.


Var_Active_Genes
3D7B
81
4
59
10G
4
12B
4



2 Code

2.1 Load Packages and functions

```
#### Imports ####

library(readxl)
library(tidyverse)
library(eulerr)

#### Max Dif function ####

max_dif <- function(vect){
  mx <- max(vect, na.rm = T)
  mn <- min(vect, na.rm = T)
```

```
  if (is.infinite(mx) | is.infinite(mn)) {
    md <- NA
  } else {
    md <- mx - mn
  }
  return(md)
}
```

2.2 Microarray Data: Red Signal

```
#### Red Signal DF ####

## Read translation table
map <- read.csv('./Data/oldnames_table.csv', stringsAsFactors = F)
excl <- "./Data/3D7_Variantome_AllData_withGam.xls"

## Import Red Signal table
red <- read_excel(excl, sheet = 4)

colnames(red)[1] <- "Old_id"

red_df <- red %>%
  select(Old_id,
         Red_12B = `Aver.2Higher1.2B.`,
         Red_10G = `Aver.2Higher10G.`,
         Red_3D7B = `Aver.2Higher3D7-B.`) %>%
  left_join(map, by='Old_id')

## Manually add gene ids that do not appear on map (we blasted the probes to assign th
red_df <- red_df %>%
  mutate(Gene_id = ifelse(Old_id == 'MAL8P1.310', 'PF3D7_0830200', Gene_id)) %>%
  mutate(Gene_id = ifelse(Old_id == 'PFI0905W', 'PF3D7_0918500', Gene_id)) %>%
  mutate(Gene_id = ifelse(Old_id == 'PFL1580W', 'PF3D7_1232700', Gene_id))

## Collapse gene ids that appear more than once (mean expression)
red_df <- red_df %>%
  select(-Old_id) %>%
  group_by(Gene_id) %>% summarize_all(list(mean))

## Filter out rows with untranslated gene ids (marked by '_oldname')
```

```
red_df <- red_df %>%
  filter(!grepl('_oldname', Gene_id))

## Transform into percentiles

red_df <- red_df %>%
  mutate(Percent_12B = (rank(Red_12B)/length(Red_12B))*100) %>%
  mutate(Percent_10G = (rank(Red_10G)/length(Red_10G))*100) %>%
  mutate(Percent_3D7B = (rank(Red_3D7B)/length(Red_3D7B))*100)

## Add max percentile dif

red_df <- red_df %>%
  mutate(MaxRedPercentDif= apply(select(., contains('Percent_')), 1, max_dif)) %>%
  mutate(MeanRedPercent = apply(select(., contains('Percent_')), 1, mean))

print(red_df, width = 200)
hist(red_df$MeanRedPercent)
```

2.3 Microarray Data: Areas

```
#### Areas DF ####

# Import Areas table

area <- read_excel(excl, sheet = 2)

colnames(area)[1] <- "Old_id"

area_df <- area %>%
  select(Old_id,
         l_12B = `left.1.2b`,
         r_12B = `right.1.2b`,
         m_12B = `mid.1.2b`,
         s_12B = `sides.1.2b`,
         l_10G = `left.10g`,
         r_10G = `right.10g`,
         m_10G = `mid.10g`,
         s_10G = `sides.10g`,
         l_3D7B = `left.3d7b`,
```

```
          r_3D7B = `right.3d7b`,
          m_3D7B = `mid.3d7b`,
          s_3D7B = `sides.3d7b`) %>%

  mutate_at(vars(-Old_id), as.numeric) %>%


  left_join(map, by='Old_id') %>%
  select(-Old_id) %>%
  group_by(Gene_id) %>% summarize_all(list(mean))


print(area_df, width = 200)

area_df <- area_df %>%
  mutate(MaxLeft = apply(select(., contains('l_')), 1, max)) %>%
  mutate(MinLeft = apply(select(., contains('l_')), 1, min)) %>%

  mutate(MaxRight = apply(select(., contains('r_')), 1, max)) %>%
  mutate(MinRight = apply(select(., contains('r_')), 1, min)) %>%

  mutate(MaxMid = apply(select(., contains('m_')), 1, max)) %>%
  mutate(MinMid = apply(select(., contains('m_')), 1, min)) %>%

  mutate(MaxSides = apply(select(., contains('s_')), 1, max)) %>%
  mutate(MinSides = apply(select(., contains('s_')), 1, min)) %>%

  mutate(DifLeft = MaxLeft - MinLeft) %>%
  mutate(DifRight = MaxRight - MinRight) %>%
  mutate(DifMid = MaxMid - MinMid) %>%
  mutate(DifSides = MaxSides - MinSides)

print(area_df, width = 200)

## Add max interval and difference

maxinterval <- area_df %>%
  select(Gene_id, contains('Dif')) %>%
  pivot_longer(-Gene_id, names_to = 'Interval', values_to = 'MaxDif') %>%
  group_by(Gene_id) %>%
```

```
  filter(rank(-MaxDif, ties.method = "first") == 1) %>%
  mutate(Interval = ifelse(is.na(MaxDif), 'No Data', Interval)) %>%
  mutate(Interval = case_when(Interval == 'DifLeft' ~ 'Left',
                              Interval == 'DifRight' ~ 'Right',
                              Interval == 'DifMid' ~ 'Mid',
                              Interval == 'DifSides' ~ 'Sides',
                              Interval == 'No Data' ~ 'No Data')) %>%
  mutate(areaFC = MaxDif/13.45)

maxinterval

area_df <- area_df %>%
  left_join(maxinterval, by = 'Gene_id')

print(area_df, width = 400)

## Select appropiate area for each gene and add max and min areas

area_df <- area_df %>%
  mutate(area_12B = case_when(
           Interval == 'Left' ~ l_12B,
           Interval == 'Right' ~ r_12B,
           Interval == 'Mid' ~ m_12B,
           Interval == 'Sides' ~ s_12B,
           Interval == 'No Data' ~ NA_real_)) %>%
  mutate(area_10G = case_when(
           Interval == 'Left' ~ l_10G,
           Interval == 'Right' ~ r_10G,
           Interval == 'Mid' ~ m_10G,
           Interval == 'Sides' ~ s_10G,
           Interval == 'No Data' ~ NA_real_)) %>%
  mutate(area_3D7B = case_when(
           Interval == 'Left' ~ l_3D7B,
           Interval == 'Right' ~ r_3D7B,
           Interval == 'Mid' ~ m_3D7B,
           Interval == 'Sides' ~ s_3D7B,
           Interval == 'No Data' ~ NA_real_)) %>%
  mutate(MaxArea = apply(select(., contains('area_')), 1, max)) %>%
  mutate(MinArea = apply(select(., contains('area_')), 1, min))
```

```r
print(area_df, width = 400)
```

2.4 Load RNA-Seq Data

```r
## #### Old approax ####
## Select max percentile directly

## otto <- read_delim("./Data/RNA_Seq_Percentiles/PlasmoDB_Otto.csv", delim=";") %>%
##   select(Gene_id = `Gene ID`, MaxPercOtto = `Max %ile (Within Chosen Samples)`)
## hoej <- read_delim("./Data/RNA_Seq_Percentiles/PlasmoDB_Hoejimakers.csv", delim=";")
##   select(Gene_id = `Gene ID`, MaxPercHoej = `Max %ile (Within Chosen Samples)`)
## toen <- read_delim("./Data/RNA_Seq_Percentiles/PlasmoDB_Toenke.csv", delim=";") %>%
##   select(Gene_id = `Gene ID`, MaxPercToen = `Max %ile (Within Chosen Samples)`)
## bart <- read_delim("./Data/RNA_Seq_Percentiles/PlasmoDB_Bartfai.csv", delim=";") %>
##   select(Gene_id = `Gene ID`, MaxPercBart = `Max %ile (Within Chosen Samples)`)


## Otto Data-Set

csv <- './Data/RNA_Seq_Percentiles/rnaseq_otto_normvals.csv'

otto <- read_delim(csv, delim=";") %>%
  select(Gene_id = `Gene ID`, contains('unique'))

otto <- otto %>%
  mutate(Max = apply(select(., contains('unique')), 1, max)) %>%
  mutate(Otto_Max_pcnt = (rank(Max)/length(Max))*100)

otto <- otto %>% select(Gene_id, Otto_Max_pcnt)
otto

hist(otto$Otto_Max_pcnt)

## Hoeijmakers Data-Set

csv <- './Data/RNA_Seq_Percentiles/rnaseq_hoeijmakers_normvals.csv'

hoeij <- read_delim(csv, delim=";") %>%
  select(Gene_id = `Gene ID`, contains('scaled'))
```

```
hoeij <- hoeij %>%
  mutate(Max = apply(select(., contains('scaled')), 1, max)) %>%
  mutate(Hoeij_Max_pcnt = (rank(Max)/length(Max))*100)

hoeij <- hoeij %>% select(Gene_id, Hoeij_Max_pcnt)
hoeij

hist(hoeij$Hoeij_Max_pcnt)

## Toenhake Data-Sets

csv <- './Data/RNA_Seq_Percentiles/rnaseq_toen_normvals.csv'

toen <- read_delim(csv, delim=",") %>%
  select(Gene_id = `Gene ID`, contains('unique'))

toen <- toen %>%
  mutate(Max = apply(select(., contains('unique')), 1, max)) %>%
  mutate(Toen_Max_pcnt = (rank(Max)/length(Max))*100)

toen <- toen %>% select(Gene_id, Toen_Max_pcnt)
toen

hist(toen$Toen_Max_pcnt)

## Bartfai Data-Sets

csv <- './Data/RNA_Seq_Percentiles/rnaseq_bartfai_normvals.csv'

bart <- read_delim(csv, delim=",") %>%
  select(Gene_id = `Gene ID`, contains('scaled'))

bart <- bart %>%
  mutate(Max = apply(select(., contains('scaled')), 1, max)) %>%
  mutate(Bart_Max_pcnt = (rank(Max)/length(Max))*100)

bart <- bart %>% select(Gene_id, Bart_Max_pcnt)
bart
```

```r
hist(bart$Bart_Max_pcnt)


## Join DF
rna_df <- otto %>%
  full_join(hoeij) %>%
  full_join(toen) %>%
  full_join(bart)

## Add mean and sd
rna_df <- rna_df %>%
  mutate(MeanPercent = apply(select(., -Gene_id), 1, mean)) %>%
  mutate(StdDevPercent = apply(select(., -Gene_id), 1, sd))


print(rna_df, width=200)

hist(rna_df$MeanPercent, breaks = 20)
hist(rna_df$StdDevPercent, breaks = 100)
```

**

2.5 Load Annotation

```r
annot_df <- read_delim('./Data/plasmoDB_geneAnnot.csv', delim = ';') %>%
  select(Gene_id = `Gene ID`,
         Gene_name = `Gene Name or Symbol`,
         Annot = `Product Description`)

print(annot_df, width=200)
```

2.6 Create Join DF

```r
print(red_df, width = 200)
print(area_df, width = 200)
print(rna_df, width = 200)

all_df <- select(red_df, Gene_id, contains('Percent'), MeanRedPercent) %>%
  full_join(select(area_df, Gene_id, Interval, contains('area')), by = 'Gene_id') %>%
  full_join(select(rna_df, Gene_id, MeanPercent), by = 'Gene_id')
```

```r
## Add Vartiant Genes information

cvg <- read_excel("./Data/CVG_list_jan2020_final.xlsx", sheet = "Final")

final_df <- cvg %>%
  select("Gene_id" = `Gene ID`, "Variant" = `Final Customized`) %>%
  right_join(all_df, by = 'Gene_id') %>%
  mutate(Variant = recode(Variant, YES = TRUE, NO = FALSE, .missing = FALSE))

print(final_df, width = 200)

## Here we create a dplyr function.
##To be able to use variables (for colnames) we needto use the special quote functions
## Colnames to use inside functions must be "enquoted" before usage and preceded by !!
## Colnames to assign must be "enquoted" first, preceded by !! and assigned by :=


## First create a col where we set categories for each gene according relative express
## For each gene: gene-min----|---mid----|----gene-max

relexprs <- function(vect){
  if (any(is.na(vect))){
    return(NA)
  } else {
    labs = c('min', 'mid', 'max')
    lab <- cut(vect, 3, labels = labs)[1]
    return(as.character(lab))
  }
}

set_relexprs <- function(df, outcol, areacol){
  outcol <- enquo(outcol)
  areacol <- enquo(areacol)
  df %>%
    mutate(!! outcol := apply(select(., !! areacol, MaxArea, MinArea), 1, relexprs))
}

final_df <- final_df %>%
  set_relexprs(rel_12B, area_12B) %>%
  set_relexprs(rel_10G, area_10G) %>%
```

```
  set_relexprs(rel_3D7B, area_3D7B)

## Add annotation
final_df <- left_join(final_df, annot_df, by = 'Gene_id')

print(final_df, width = 200)
```

2.7 Create Lists according to thresholds

```
print(final_df, width = 200)

th_rnapcnt <- 25
th_redpcnt <- 25
th_redrescue <- 40
th_areaFC <- 1

## Here we create a dplyr function.
##To be able to use variables (for colnames) we needto use the special quote functions
## Colnames to use inside functions must be "enquoted" before usage and preceded by !!
## Colnames to assign must be "enquoted" first, preceded by !! and assigned by :=

set_state <- function(df, statecol, redcol, relcol){

  statecol <- enquo(statecol)
  redcol <- enquo(redcol)
  relcol <- enquo(relcol)

  df <- df %>%
    mutate(!! statecol := case_when(
                ## Actiu
                !Variant &
                areaFC < th_areaFC &
                MeanPercent >= th_rnapcnt ~ 'Active',

                ## Inactiu
                !Variant &
                areaFC < th_areaFC &
                MeanPercent < th_rnapcnt ~ 'Inactive',

                ## No variant amb FC
```

```
!Variant &
areaFC > th_areaFC &
MeanPercent >= th_rnapcnt ~ 'Active_FC',

!Variant &
areaFC > th_areaFC &
MeanPercent < th_rnapcnt ~ 'Inactive_FC',

## Var actiu
Variant &
areaFC < th_areaFC &
MeanPercent >= th_rnapcnt ~ 'Var_Active_noFC', # noFC

Variant &
areaFC < th_areaFC &
MeanPercent < th_rnapcnt &
MeanRedPercent >= th_redrescue ~ 'Var_Active_noFC_rescued', # noFC, re

Variant &
areaFC >= th_areaFC &
!! redcol >= th_redpcnt &
!! relcol == 'max' ~ 'Var_Active_FC', # Variant, FC, redpcnt, max

Variant &
areaFC >= th_areaFC &
!! redcol >= th_redpcnt &
!! relcol == 'mid' ~ 'Var_Semiactive_FC', # Variant, FC, redpcnt, mid

## Var repressed
Variant &
areaFC < th_areaFC &
MeanPercent < th_rnapcnt &
MeanRedPercent < th_redrescue ~ 'Var_Repressed_noFC', # noFC, noRescue

Variant &
areaFC >= th_areaFC &
!! redcol >= th_redpcnt &
!! relcol == 'min' ~ 'Var_Repressed_FC', # Variant, FC, redpcnt, min

Variant &
```

```
                areaFC >= th_areaFC &
                !! redcol < th_redpcnt ~ 'Var_Repressed_FC_noredpcnt', # Variant, FC,

                ## Not settable
                is.na(areaFC) | is.na(MeanPercent) ~ 'Not_settable',

                TRUE ~ 'Wrong!'))

  ## The 'TRUE ~ ...' handles rows that do not match any of previous patterns.
  ## Here we use it to make sure all rows are set (no "Wrong!" appearing)

  return(df)
}


set_category <- function(df, statecol, categorycol){

  statecol <- enquo(statecol)
  categorycol <- enquo(categorycol)

  df <- df %>%
    mutate(!! categorycol := case_when(
                !! statecol == 'Active' ~ 'Active',
                !! statecol == 'Inactive' ~ 'Inactive',
                !! statecol == 'Active_FC' ~ 'Active',
                !! statecol == 'Inactive_FC' ~ 'Inactive',
                !! statecol == 'Var_Active_noFC' ~ 'Var_Active',
                !! statecol == 'Var_Active_noFC_rescued' ~ 'Var_Active',
                !! statecol == 'Var_Repressed_noFC' ~ 'Var_Repressed',
                !! statecol == 'Var_Active_FC' ~ 'Var_Active',
                !! statecol == 'Var_Semiactive_FC' ~ 'Var_Semiactive',
                !! statecol == 'Var_Repressed_FC' ~ 'Var_Repressed',
                !! statecol == 'Var_Repressed_FC_noredpcnt' ~ 'Var_Repressed',
                !! statecol == 'Not_settable' ~ 'Not_settable',
                TRUE ~ 'No_Category!'))
  return(df)
}

## We now set each gene to it's state
```

```r
state_df <- final_df %>%
  set_state(state_12B, Percent_12B, rel_12B) %>%
  set_state(state_10G, Percent_10G, rel_10G) %>%
  set_state(state_3D7B, Percent_3D7B, rel_3D7B) %>%
  set_category(state_12B, category_12B) %>%
  set_category(state_10G, category_10G) %>%
  set_category(state_3D7B, category_3D7B)

print(state_df, width = 400)
```

2.8 Some checks and results

```r
## We check no rows are set to "Wrong!"
state_df %>%
  filter(state_12B == 'Wrong!' | state_10G == 'Wrong!' | state_3D7B == 'Wrong!') %>%
  print(width = 400)

state_df %>%
  filter(category_12B == 'No_Category' |
         category_10G == 'No_Category' |
         category_3D7B == 'No_Category')

## Save results
write.csv(state_df, './Results_Tables/state_df_rna25_red25_reddif0_area1.csv')

## Create a table with number of each state per strain
state_table <-  bind_rows(table(state_df$state_12B),
                          table(state_df$state_10G),
                          table(state_df$state_3D7B)) %>%
  replace_na(list(Var_Semiactive = 0)) %>%
  mutate(Strain = c('12B', '10G', '3D7B')) %>%
  select(Strain, everything())

## Create a table with differences between 12B and 10G
dif12B_10G <- state_df %>%
  filter(state_12B != state_10G) %>%
  select(Gene_id, contains('12B'), contains('10G'), Gene_name, Annot)

## Check Clags
clags <- state_df %>%
```

```
  filter(Gene_id == 'PF3D7_0302500' | Gene_id == 'PF3D7_0302200')

write.csv(clags, './Results_Tables/clag_genes.csv')

print(state_table, width = 200)
summary(rna_df)
```

2.9 Comparison of 12B vs 10G differences

```
excl <- "./Data/10Gvs1p2B.xlsx"

## Import 12B vs 10G differences by transcription table
trans_difs <- read_delim('./Data/transDif_12Bvs10G.csv', delim = ';') %>%
  rename(Old_id = X1) %>%
  left_join(map, by = 'Old_id')

print(trans_difs, width = 400)

trans_difs %>%
  select(Old_id, Gene_id)

dif12B_10G

table(trans_difs$Gene_id %in% dif12B_10G$Gene_id)
table(dif12B_10G$Gene_id %in% trans_difs$Gene_id)

allids <- unique(c(dif12B_10G$Gene_id, trans_difs$Gene_id))

compare_12Bvs10G <- state_df %>%
  filter(Gene_id %in% allids) %>%
  select(Gene_id,
         Variant, areaFC,
         MeanRedPercent,
         contains('12B'),
         contains('10G'),
         Gene_name,
         Annot) %>%
  mutate(TransDif = Gene_id %in% trans_difs$Gene_id) %>%
  mutate(Dif_state = category_12B != category_10G)
```

```
print(compare_12Bvs10G, width = 400)
write.csv(compare_12Bvs10G, './Results_Tables/gens_dif12B_10G.csv')
```

2.10 Venn Diagram of results

```
makeIntersects <- function(a,b,c){

  a_b <- intersect(a, b)
  a_c <- intersect(a, c)
  b_c <- intersect(b, c)
  a_b_c <- intersect(a_b, c)

  abc <- a_b_c
  ab <- a_b[!a_b %in% a_b_c]
  ac <- a_c[!a_c %in% a_b_c]
  bc <- b_c[!b_c %in% a_b_c]

  a <- a[!a %in% ab & !a %in% ac & !a %in% abc]
  b <- b[!b %in% ab & !b %in% bc & !b %in% abc]
  c <- c[!c %in% ac & !c %in% bc & !c %in% abc]

  return(list(a = a, b = b, c = c, ab = ab, bc = bc, ac = ac, abc = abc))

}

customEuler <- function(a,b,c, name){

  intersects <- makeIntersects(a,b,c)
  areas <- lapply(intersects, function(x) length(x))

  fit <- euler(c(A=areas$a, B=areas$b, C=areas$c,
                 "A&B"=areas$ab, "A&C"=areas$ac, "B&C"=areas$bc,
                 "A&B&C" = areas$abc))

  d <- plot(fit, fills = list(fill = c("#619CFF", "#F8766D", "#00BA38"), alpha = 0.5),
            edges = list(lwd = 0.1), quantities = list(quantities = T),
            labels = list(labels=c("12B", "10G", "3D7B")),
            main = name)

  ggsave(d, filename = paste0('./Plots/', "venn_", name, ".png"),
```

```
        device = "png", width = 10, height = 10, units = "cm")

  plot(d)
  print(fit)
}


## Var active

va_12B <- state_df %>%
  filter(category_12B == "Var_Active") %>%
  select(Gene_id) %>%
  pull()

va_10G <- state_df %>%
  filter(category_10G == "Var_Active") %>%
  select(Gene_id) %>%
  pull()

va_3D7B <- state_df %>%
  filter(category_3D7B == "Var_Active") %>%
  select(Gene_id) %>%
  pull()


customEuler(va_12B, va_10G, va_3D7B, 'Var_Active_Genes')

## Var inactive

vi_12B <- state_df %>%
  filter(category_12B == "Var_Repressed") %>%
  select(Gene_id) %>%
  pull()

vi_10G <- state_df %>%
  filter(category_10G == "Var_Repressed") %>%
  select(Gene_id) %>%
  pull()

vi_3D7B <- state_df %>%
```

```
  filter(category_3D7B == "Var_Repressed") %>%
  select(Gene_id) %>%
  pull()


customEuler(vi_12B, vi_10G, vi_3D7B, 'Var_Repressed_Genes')

## Same category
## We fuse the gene_id with it's state so that genes with same state will be exactly t

print(state_df, width = 400)

genestate <- state_df %>%
  mutate(gs_12B = paste(Gene_id, category_12B, sep = "_")) %>%
  mutate(gs_10G = paste(Gene_id, category_10G, sep = "_")) %>%
  mutate(gs_3D7B = paste(Gene_id, category_3D7B, sep = "_")) %>%
  select(Gene_id,
         category_12B, category_10G, category_3D7B,
         gs_12B, gs_10G, gs_3D7B)

customEuler(genestate$gs_12B, genestate$gs_10G, genestate$gs_3D7B, 'Same_State')

x <- makeIntersects(genestate$gs_12B, genestate$gs_10G, genestate$gs_3D7B)}
```