Creation of active gene-lists

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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
 - Regular
 - Variant Active
 - Variant Repressed
- Inactive
- Not-Settable

1.1 Microarray Data: Red Signal

We will 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
1	mal_mito_3	22579.33333	36436.73333	30636.82500	96.0335622
2	MAL13P1.415_oldname	770.82083	702.22292	640.11667	21.3196034
3	MAL13P1.65_oldname	111.33333	87.05833	91.05833	6.2166285
4	MAL7P1.142_oldname	5924.44167	5194.40000	5114.63333	75.4767353
5	MAL8P1.310_oldname	37.21250	35.37917	33.24167	0.8581236
6	MAL8P1.90_oldname	80.55417	46.18333	54.64167	4.1952708
	Percent_10G Percent	_3D7B MaxRedI	PercentDif		
1	98.474447 97.88	32952	2.4408848		
2	20.861937 18.45	91915	2.8604119		
3	5.053394 4.51	94508	1.6971777		
4	72.444699 71.72	00610	3.7566743		
5	1.115561 0.59	11518	0.5244088		
6	2.002288 2.25	01907	2.1929825		

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

```
1_10G
               Gene_id
                           1_12B
                                      r_12B
                                                 m_12B
                                                           s_12B
                                                                               r_10G
           mal_mito_3 30.592496 61.080128 49.676556 41.99607 25.372470 62.38873
1
                                   8.488971
                                              1.289779 12.62246
2 MAL13P1.415_oldname
                        5.423269
                                                                  6.117132 10.59524
   MAL13P1.65_oldname 18.322430
                                         NA 17.593468
                                                              NA 14.071128
                                                                                  NA
   MAL7P1.142_oldname
                        9.389247
                                  12.807814 10.340803 11.85626 13.661078 14.52676
   MAL8P1.310_oldname
5
                               NA
                                         NA
                                                    NA
                                                              NA
                                                                        NA
                                                                                  NA
6
    MAL8P1.90_oldname
                                                                        NA
                               NA
                                         NA
                                                    NA
                                                              NA
                                                                                  NA
      m_10G
                s_10G
                         1_3D7B
                                   r_3D7B
                                              m_3D7B
                                                        s_3D7B
                                                                  MaxLeft
                                                                             MinLeft
  49.805504 37.95570 25.484634 62.83441 50.462696 37.856349 30.592496 25.372470
   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
  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
  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
                          Left 5.220025 0.3881060 30.592496 25.37247 25.484634
1 0.7861401 4.139719
 2.4019733 4.425700
                         Sides 4.425700 0.3290483 12.622460 13.03616
                                                                         8.610459
3
                          Left 5.262196 0.3912413 18.322430 14.07113 19.333324
         NA
                   NA
 3.0608067 6.383199
                          Left 6.562046 0.4878845
                                                     9.389247 13.66108
4
                                                                         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
  13.66108
            7.099032
5
        NA
                   NA
6
        NA
                   NA
```

1.3 Load RNA-Seq Data

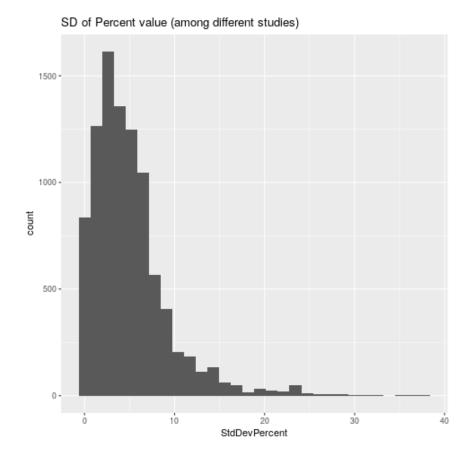
We will use publicly available data from PlasmoDB to create a reference expression percentile for each gene. All data-sets are from RNA-Seq studies in the 3D7 strain. We are using 4 different data-sets:

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

RNA-Seq DataFrame

	Gene_id	${\tt MaxPercOtto}$	${\tt MaxPercHoej}$	${\tt MaxPercToen}$	${\tt MaxPercBart}$	MeanPercent
1	PF3D7_0100100	57.2	54.3	33.9	31.7	44.275
2	PF3D7_0100200	29.4	50.5	26.6	36.0	35.625
3	PF3D7_0100300	34.2	8.7	7.7	7.4	14.500
4	PF3D7_0100400	50.3	18.3	11.3	37.4	29.325
5	PF3D7_0100500	49.7	11.4	14.0	32.5	26.900
6	PF3D7_0100600	18.5	7.8	2.3	12.1	10.175
	${\tt StdDevPercent}$					
1	11.546942					
2	9.241313					
3	11.383980					
4	15.424068					
5	15.474657					
6	5.930167					

We plot the standard deviation of the percentile values among different studies 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_id Variant Percent_12B Percent_10G Percent_3D7B mal_mito_3 FALSE 96.0335622 98.474447 97.8832952

```
2 MAL13P1.415_oldname
                         FALSE
                                 21.3196034
                                               20.861937
                                                            18.4591915
   MAL13P1.65_oldname
                                                5.053394
                                                             4.5194508
                         FALSE
                                  6.2166285
   MAL7P1.142_oldname
                         FALSE
                                 75.4767353
                                               72.444699
                                                            71.7200610
                         FALSE
                                                             0.5911518
   MAL8P1.310_oldname
                                  0.8581236
                                                1.115561
    MAL8P1.90_oldname
                         FALSE
                                                2.002288
                                                             2.2501907
6
                                  4.1952708
                                          area_12B area_10G area_3D7B
  MaxRedPercentDif Interval
                                 areaFC
                                                                        MaxArea
1
         2.4408848
                        Left 0.3881060 30.592496 25.37247 25.484634 30.59250
2
         2.8604119
                       Sides 0.3290483 12.622460 13.03616
                                                              8.610459 13.03616
3
         1.6971777
                        Left 0.3912413 18.322430 14.07113 19.333324 19.33332
4
         3.7566743
                        Left 0.4878845
                                         9.389247 13.66108
                                                              7.099032 13.66108
5
         0.5244088
                     No Data
                                     NA
                                                NA
                                                          NA
                                                                    NA
                                                                              NA
6
                                                NA
                                                          NA
                                                                    NA
         2.1929825
                     No Data
                                     NA
                                                                              NA
    MinArea MeanPercent rel_12B rel_10G rel_3D7B
                  95.975
1 25.372470
                              max
                                      min
                                                min
   8.610459
                      NA
                                                min
                              max
                                      max
3 14.071128
                      NA
                              max
                                      min
                                                max
   7.099032
                      NA
                              mid
                                      max
                                                min
5
         NA
                                       NA
                      NA
                               NA
                                                 NA
6
                      NA
         NA
                               NA
                                       NA
                                                 NA
```

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 Percentile (by sample) for "rescuing": 40%
- (red_dif) Red Signal percentile diference: 0 (we are currently not using it but it is set, just in case)
- (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:

- Active :
 - Regular
 - 1. Non-Variant
 - 2. > rna pcnt
 - Variant Active
 - * Case 1 (no area fc):
 - 1. Variant
 - 2. < area fc
 - 3. > rna pcnt
 - * Case 2 (no area_fc, rescued):
 - 1. Variant
 - $2. < area_fc$
 - 3. < rna pcnt
 - 4. > red rescue
 - * Case 3 (area fc, max):
 - 1. Variant
 - 2. > area fc
 - 3. > red dif
 - 4. > red pcnt
 - 5. $rel_exprs = max$
 - * Case 4 (area fc, mid):
 - 1. Variant
 - 2. > area fc
 - 3. > red dif
 - 4. > red pcnt
 - $5. \text{ rel} \text{_exprs} = \text{mid}$
 - Variant Repressed:
 - * Case 1 (no area fc, no rescue):
 - 1. Variant

- 2. < area fc
- $3. < rna_pcnt$
- $4.\ < {\rm red_rescue}$
- * Case 2 (area_fc, rel_exprs = mid)
 - 1. Variant
 - 2. > area fc
 - $3.~>{\rm red_dif}$
 - $4. > {\rm red_pcnt}$
 - 5. $rel_exprs = min$
- * Case 3 (area_fc, low red_pcnt)
 - 1. Variant
 - $2.\ > area_fc$
 - $3. > \text{red_dif}$
 - $4. < red_pcnt$

• Inactive:

- 1. Non-Variant
- 2. < rna pcnt
- Not Settable:
 - 1. area_fc OR rna_pcnt are not set (NA).

Using these classification logic we get the following table: Gene-State DataFrame

	Gene_id	Variant	Percent_12B	Percent_10G	Percent_3D7B	
1	mal_mito_3	FALSE	96.0335622	98.474447	97.8832952	
2	MAL13P1.415_oldname	FALSE	21.3196034	20.861937	18.4591915	
3	MAL13P1.65_oldname	FALSE	6.2166285	5.053394	4.5194508	
4	MAL7P1.142_oldname	FALSE	75.4767353	72.444699	71.7200610	
5	MAL8P1.310_oldname	FALSE	0.8581236	1.115561	0.5911518	
6	MAL8P1.90_oldname	FALSE	4.1952708	2.002288	2.2501907	
	MaxRedPercentDif In	terval	areaFC ar	ea_12B area_3	10G area_3D7B	${\tt MaxArea}$
1	2.4408848	Left 0	.3881060 30.	592496 25.372	247 25.484634	30.59250
2	2.8604119	Sides 0	.3290483 12.	622460 13.036	8.610459	13.03616
3	1.6971777	Left. 0	3912413 18	322430 14 07	113 19 333324	19.33332

4	3.7	566743	Left 0.4	878845	9.389247	13.66108 7.0	99032	13.66108
5	0.5	244088 No	Data	NA	NA	NA	NA	NA
6	2.1	929825 No	Data	NA	NA	NA	NA	NA
	MinArea	MeanPercent	rel_12B	rel_10G	rel_3D7B	state_12B	st	ate_10G
1	25.372470	95.975	5 max	min	min	Active		Active
2	8.610459	NA	nax	max	min	Not_settable	Not_s	ettable
3	14.071128	NA	nax	min	max	Not_settable	Not_s	ettable
4	7.099032	NA	nid mid	max	min	Not_settable	Not_s	ettable
5	NA	NA	NA NA	NA	NA	Not_settable	Not_s	ettable
6	NA	NA	NA NA	NA	NA	Not_settable	Not_s	ettable
	state_3D	7B						

- 1 Active
- 2 Not_settable
- 3 Not_settable
- 4 Not_settable
- 5 Not_settable
- 6 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	Inactive	$Not_settable$	Var_Active	Var_Repressed	Var_Semiactive
12B	8302	306	246	167	265	1
10G	8302	306	246	164	269	0
3D7B	8302	306	246	249	184	0

And this are the Clag genes States Table

Gene_id Variant Percent_12B Percent_10G Percent_3D7B MaxRedPercentDif 1 PF3D7_0302200 78.69947 99.58047 42.37223 57.20824 TRUE 2 PF3D7_0302500 TRUE 98.81770 84.40122 98.98932 14.58810 Interval areaFC area_12B area_10G area_3D7B MaxArea MinArea MeanPercent 1 Right 4.823089 37.32948 88.19577 23.32522 88.19577 23.32522 85.35 Right 3.452270 100.12065 53.68762 99.58767 100.12065 53.68762 94.90 rel_12B rel_10G rel_3D7B state_12B state_10G state_3D7B 1 Var_Active Var_Repressed min maxmin Var_Repressed 2 max min max Var_Active Var_Repressed Var_Active

2 Code

Imports

2.1 Load Packages and functions

```
library(readxl)
library(tidyverse)
#### Max Dif function ####
max_dif <- function(vect){</pre>
  mx <- max(vect, na.rm = T)
  mn <- min(vect, na.rm = T)</pre>
  if (is.infinite(mx) | is.infinite(mn)) {
    md < - NA
  } else {
    md < -mx - mn
  return(md)
}
     Microarray Data: Red Signal
#### Red Signal DF ####
## Read translation table
map <- read.csv('./Data/oldnames_table.csv')</pre>
excl <- "./Data/3D7_Variantome_AllData_withGam.xls"</pre>
## Import Red Signal table
red <- read_excel(excl, sheet = 4)</pre>
colnames(red)[1] <- "Old_id"</pre>
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') %>%
```

```
select(-Old_id) %>%
  group_by(Gene_id) %>% summarize_all(list(mean))
## 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))
print(red_df, width = 200)
    Microarray Data: Areas
#### Areas DF ####
# Import Areas table
area <- read_excel(excl, sheet = 2)</pre>
colnames(area)[1] <- "Old_id"</pre>
area_df <- area %>%
  select(Old_id,
         1_{12B} = [1.1.2b],
         r_12B = right.1.2b,
         m_12B = mid.1.2b,
         s_12B = sides.1.2b,
         l_10G = \left[1.10g\right],
         r_10G = right.10g,
         m_10G = mid.10g,
         s_10G = `sides.10g`,
         1_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' ~ 1_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' ~ 1_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))
print(area_df, width = 400)
```

2.4 Load RNA-Seq Data

```
#### Load Data-Sets ####
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)`)
## Join DF
rna_df <- full_join(otto, hoej) %>%
  full_join(hoej) %>%
  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)
2.5
    Create Join DF
red_df
print(area_df, width = 200)
rna_df
all_df <- select(red_df, Gene_id, contains('Percent')) %>%
  full_join(select(area_df, Gene_id, Interval, contains('area')), by = 'Gene_id') %>%
  full_join(select(rna_df, Gene_id, MeanPercent), by = 'Gene_id')
## Add Vartiant Genes information
cvg <- read_excel("./Data/CVG_list_jan2020_final.xlsx", sheet = "Final")</pre>
```

```
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){</pre>
  if (any(is.na(vect))){
    return(NA)
  } else {
    labs = c('min', 'mid', 'max')
    lab <- cut(vect, 3, labels = labs)[1]</pre>
    return(as.character(lab))
  }
}
set_relexprs <- function(df, outcol, areacol){</pre>
  outcol <- enquo(outcol)</pre>
  areacol <- enquo(areacol)</pre>
    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)
print(final_df, width = 200)
```

2.6 Create Lists according to thresholds

```
print(final_df, width = 200)
th_rnapcnt <- 25
th_redpcnt <- 25
th_redrescue <- 40
th_red_difpcnt <- 0</pre>
th_areaFC <- 1
## Here we create a dplyr function.
##To be able to use variables (for colnames) we need to 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){</pre>
  statecol <- enquo(statecol)</pre>
  redcol <- enquo(redcol)</pre>
  relcol <- enquo(relcol)</pre>
  df <- df %>%
    mutate(!! statecol := case_when(
                 ## Actiu
                 !Variant & MeanPercent >= th_rnapcnt ~ 'Active',
                 ## Inactiu
                 !Variant & MeanPercent < th_rnapcnt \tilde{\ } 'Inactive',
                 ## Var actiu
                 Variant &
                 areaFC < th_areaFC &
                MeanPercent >= th_rnapcnt ~ 'Var_Active', # noFC
                Variant &
                 areaFC < th_areaFC &
                MeanPercent < th_rnapcnt &</pre>
                 !! redcol >= th_redrescue ~ 'Var_Active', # noFC, rescued
                 Variant &
                 areaFC >= th_areaFC &
```

```
MaxRedPercentDif >= th_red_difpcnt &
                !! redcol >= th_redpcnt &
                !! relcol == 'max' ~ 'Var_Active', # Variant, FC, redpcnt, max
                Variant &
                areaFC >= th_areaFC &
                MaxRedPercentDif >= th_red_difpcnt &
                !! redcol >= th_redpcnt &
                !! relcol == 'mid' ~ 'Var_Semiactive', # Variant, FC, redpcnt, mid
                ## Var repressed
                Variant &
                areaFC < th_areaFC &</pre>
                MeanPercent < th_rnapcnt &</pre>
                !! redcol < th_redrescue ~ 'Var_Repressed', # noFC, noRescued
                Variant &
                areaFC >= th_areaFC &
                MaxRedPercentDif >= th_red_difpcnt &
                !! redcol >= th_redpcnt &
                !! relcol == 'min' ~ 'Var_Repressed', # Variant, FC, redpcnt, min
                Variant &
                areaFC >= th_areaFC &
                MaxRedPercentDif >= th_red_difpcnt &
                !! redcol < th_redpcnt ~ 'Var_Repressed', # Variant, FC, NOredpcnt
                ## 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)
}
## We now set each gene to it's state
```

```
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)
## We check no rows are set to "Wrong!"
state_df %>%
  filter(state_12B == 'Wrong!' | state_10G == 'Wrong!' | state_3D7B == 'Wrong!') %>%
  print(width = 200)
## 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$state_12B),</pre>
                          table(state_df$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(contains('12B'), contains('10G'))
write.csv(dif12B_10G, './Results_Tables/gens_dif12B_10G.csv')
## Check Clags
clags <- state_df %>%
  filter(Gene_id == 'PF3D7_0302500' | Gene_id == 'PF3D7_0302200')
 write.csv(clags, './Results_Tables/clag_genes.csv')
```