

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* genome a expression state. We will define 5 possible expression states:

- Active
 - Active (no-variant genes)
 - Variant Active
 - Variant Semiactive (vairant genes with an intermediate state)
- Inactive (no-variant genes)
 - Variant Repressed
- 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	MaxRedPercent		
1	97.8800611	2.425516	97.4662592	98.4721161		
2	11.1153552	5.423988	9.2564298	11.1153552		
3	19.9006875	25.744843	17.8889738	29.7555386		
4	81.3407181	68.773873	35.6315253	81.3407181		
5	8.0595875	8.842628	6.3343519	9.8930481		
6	0.2387319	0.210084	0.3023937	0.4392666		

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 maximum 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 expression percentile for each gene. All data-sets are from RNA-Seq studies in the 3D7 strain. We are using 4 different data-sets:

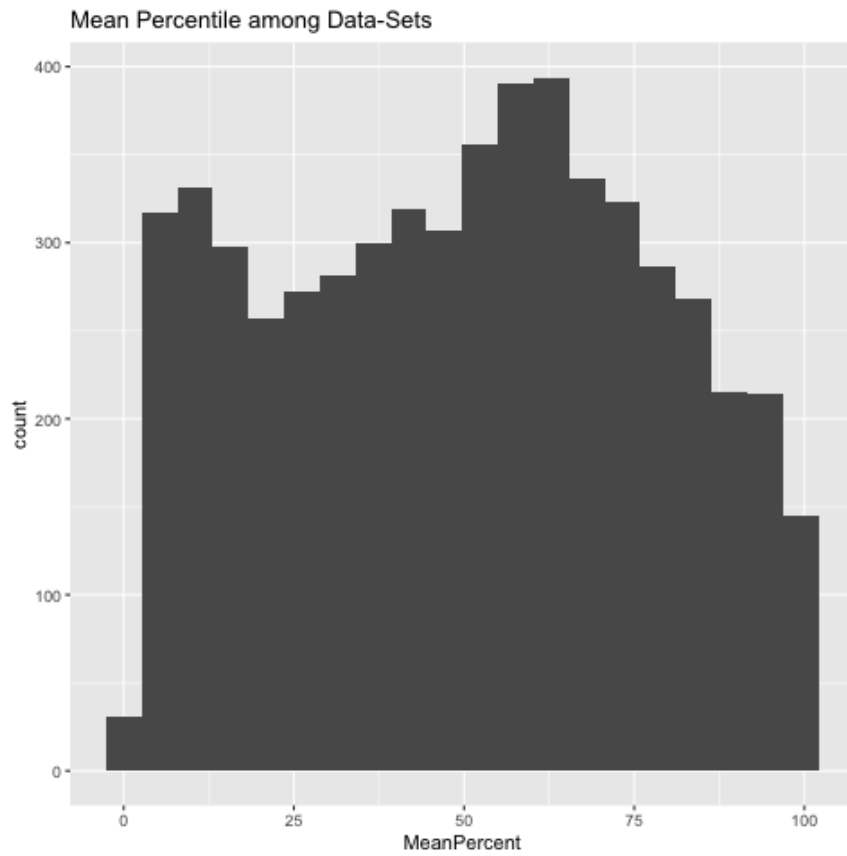
- Otto et.al.
- Hoeijmakers et.al.
- Toenhake et.al.
- Bartfai et.al.

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

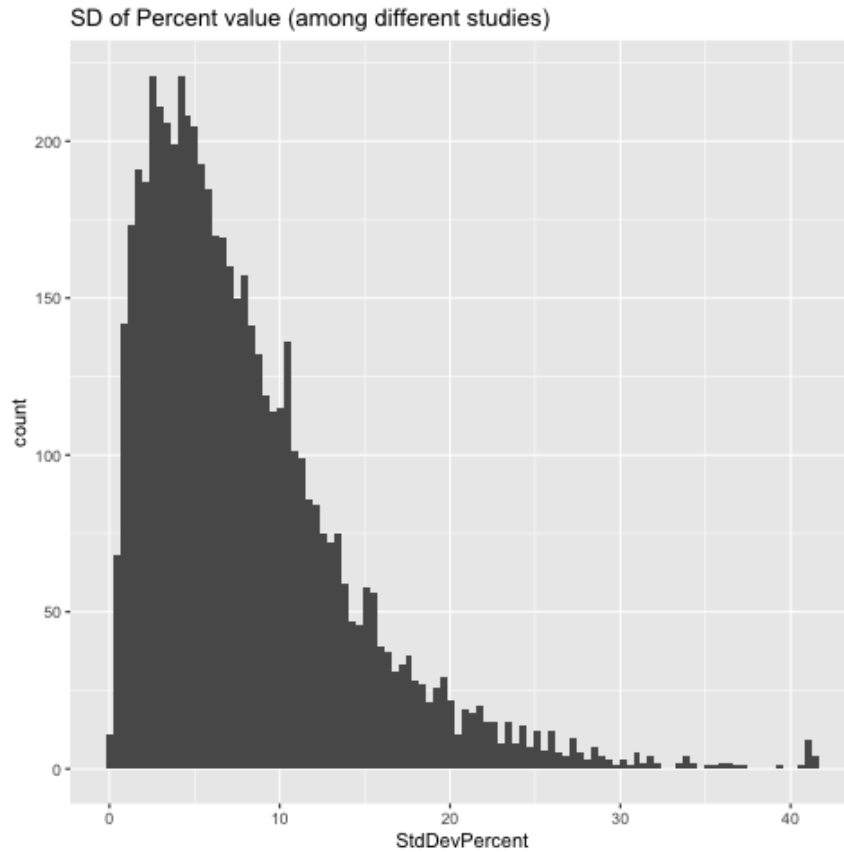
RNA-Seq DataFrame

	Gene_id	Otto_Max_pcmt	Hoeij_Max_pcmt	Toen_Max_pcmt	Bart_Max_pcmt
1	mal_mito_1	97.695933	66.259382	69.20929	80.520161
2	mal_mito_2	98.079944	88.776401	85.12829	94.728574
3	mal_mito_3	97.835573	92.249956	89.99825	90.190260
4	PF3D7_0100100	26.104032	24.855996	17.57724	18.240531
5	PF3D7_0100200	7.366032	25.693838	13.83313	18.764182
6	PF3D7_0100300	22.045732	3.587013	2.46989	1.885146
	MeanPercent	StdDevPercent			
1	78.421190	12.335823			
2	91.678303	5.040060			
3	92.568511	3.166454			
4	21.694449	3.818402			
5	16.414296	6.711285			
6	7.496945	8.421970			

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 previous data we create a table that will let us classify the genes according to their expression state.

To classify genes, we add a column for each strain where 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	PF3D7_0100100	TRUE	5.6913675	10.9625668	11.1153552	5.423988
2	PF3D7_0100200	TRUE	29.7555386	4.0106952	19.9006875	25.744843

3	PF3D7_0100300	TRUE	12.5668449	12.9870130	81.3407181	68.773873	
4	PF3D7_0100400	TRUE	9.8930481	1.0504202	8.0595875	8.842628	
5	PF3D7_0100500	TRUE	NA	NA	NA	NA	
6	PF3D7_0100600	TRUE	0.2291826	0.4392666	0.2387319	0.210084	
	MeanRedPercent	MaxRedPercent	Interval	areaFC	area_12B	area_10G	area_3D7B
1	9.2564298	11.1153552	Left	0.8879945	31.76998	43.713503	38.29839
2	17.8889738	29.7555386	Right	3.5015562	52.33320	5.237266	43.02460
3	35.6315253	81.3407181	Left	3.3957657	31.78908	33.073957	77.46213
4	6.3343519	9.8930481	Left	2.0671154	23.21846	7.706832	35.50954
5	NA	NA	<				
	NA	NA	NA	NA			
6	0.3023937	0.4392666	No Data	NA	NA	NA	NA
	MaxArea	MinArea	MeanPercent	rel_12B	rel_10G	rel_3D7B	Gene_name
1	43.71350	31.769976	21.694449	min	max	mid	VAR
2	52.33320	5.237266	16.414296	max	min	max	RIF
3	77.46213	31.789080	7.496945	min	min	max	VAR
4	35.50954	7.706832	14.607698	mid	min	max	RIF
5	NA	NA	18.975825	NA	NA	NA	null
6	NA	NA	4.789230	NA	NA	NA	RIF
							Annot
1							erythrocyte membrane protein 1, PfEMP1
2							rifin
3							erythrocyte membrane protein 1, PfEMP1
4							rifin
5							erythrocyte membrane protein 1 (PfEMP1), exon 1, pseudogene
6							rifin

1.5 Create Lists according to thresholds

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

We have set the following thresholds:

- RNA-Seq mean percentile: 25%
- Red Signal Mean Percentile : 25%
- Red Signal Percentile for "rescuing": 40%
- Red Signal Percentile for "downgrading": 15%
- Area log2 Fold-Change: 1

- Red Signal Percentile Difference: 30%

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

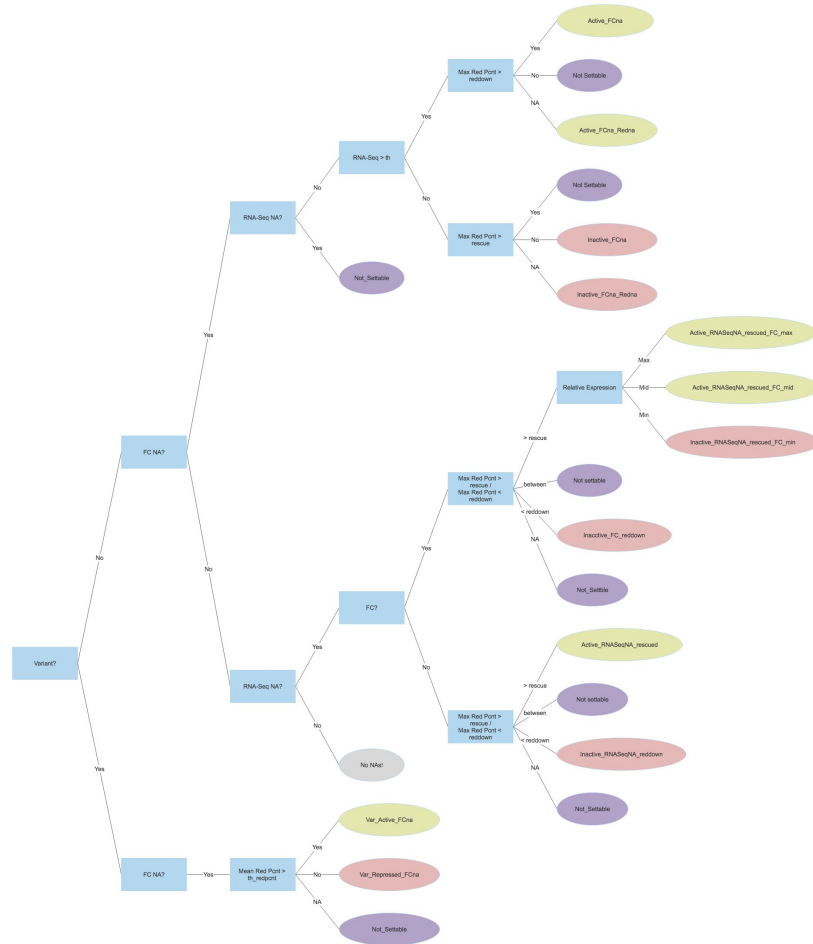
- Variant: a column stating if gene is variant/non-variant
- Relative Expression (by strain): a column where each gene is set to min/mid/max according to its expression level relative to the other strains.

We will have the following categories with the following logic:



Make bigger

Genes that have NA values in any of the classification columns are classified according to the following tree.



Make bigger

Using this criteria we construct the following DataFrame:

Gene-State DataFrame

	Gene_id	Variant	Percent_12B	Percent_10G	Percent_3D7B	MaxRedPercentDif
1	PF3D7_0100100	TRUE	5.6913675	10.9625668	11.1153552	5.423988
2	PF3D7_0100200	TRUE	29.7555386	4.0106952	19.9006875	25.744843
3	PF3D7_0100300	TRUE	12.5668449	12.9870130	81.3407181	68.773873
4	PF3D7_0100400	TRUE	9.8930481	1.0504202	8.0595875	8.842628

5	PF3D7_0100500	TRUE	NA	NA	NA	NA	
6	PF3D7_0100600	TRUE	0.2291826	0.4392666	0.2387319	0.210084	
	MeanRedPercent	MaxRedPercent	Interval	areaFC	area_12B	area_10G	area_3D7B
1	9.2564298	11.1153552	Left	0.8879945	31.76998	43.713503	38.29839
2	17.8889738	29.7555386	Right	3.5015562	52.33320	5.237266	43.02460
3	35.6315253	81.3407181	Left	3.3957657	31.78908	33.073957	77.46213
4	6.3343519	9.8930481	Left	2.0671154	23.21846	7.706832	35.50954
5	NA	NA	<				
	NA	NA	NA	NA			
6	0.3023937	0.4392666	No Data	NA	NA	NA	NA
	MaxArea	MinArea	MeanPercent	rel_12B	rel_10G	rel_3D7B	Gene_name
1	43.71350	31.769976	21.694449	min	max	mid	VAR
2	52.33320	5.237266	16.414296	max	min	max	RIF
3	77.46213	31.789080	7.496945	min	min	max	VAR
4	35.50954	7.706832	14.607698	mid	min	max	RIF
5	NA	NA	18.975825	NA	NA	NA	null
6	NA	NA	4.789230	NA	NA	NA	RIF
							Annot
1							erythrocyte membrane protein 1, PfEMP1
2							rifin
3							erythrocyte membrane protein 1, PfEMP1
4							rifin
5							erythrocyte membrane protein 1 (PfEMP1), exon 1, pseudogene
6							rifin
		state_12B		state_10G		state_3D7B	
1		Var_Repressed_noFC		Var_Repressed_noFC		Var_Repressed_noFC	
2		Var_Active_FC		Var_Repressed_FC		Var_Active_FC	
3		Var_Repressed_FC		Var_Repressed_FC		Var_Active_FC	
4		Var_Repressed_FC_reddown		Var_Repressed_FC_reddown		Var_Repressed_FC_reddown	
5		Not_Settable		Not_Settable		Not_Settable	
6		Var_Repressed_FCna		Var_Repressed_FCna		Var_Repressed_FCna	
		category_12B		category_10G		category_3D7B	
1		Var_Repressed		Var_Repressed		Var_Repressed	
2		Var_Active		Var_Repressed		Var_Active	
3		Var_Repressed		Var_Repressed		Var_Active	
4		Var_Repressed		Var_Repressed		Var_Repressed	
5		Not_Settable		Not_Settable		Not_Settable	
6		Var_Repressed		Var_Repressed		Var_Repressed	

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	Active_FC_Dif_max	Active_FCna	Active_FCna_Redna
1	12B	3954	23	2	2	191
2	10G	3954	23	2	2	191
3	3D7B	3954	23	2	2	191
	Active_rescued	Active_RNASeqNA_rescued	Inactive	Inactive_FC		
1	69		6	644	36	
2	69		6	644	36	
3	69		6	644	36	
	Inactive_FC_Dif_min	Inactive_FC_reddown	Inactive_FCna	Inactive_FCna_Redna		
1	2	2	30	89		
2	2	2	30	89		
3	2	2	30	89		
	Inactive_reddown	Not_Settable	Var_Active_FC	Var_Active_noFC	Var_Repressed_FC	
1	26	150	13	58	73	
2	26	150	8	58	77	
3	26	150	80	58	6	
	Var_Repressed_FC_reddown	Var_Repressed_FCna	Var_Repressed_noFC			
1	64	93	130			
2	64	93	130			
3	64	93	130			
	Var_Semiactive_FC					
1	3					
2	4					
3	3					

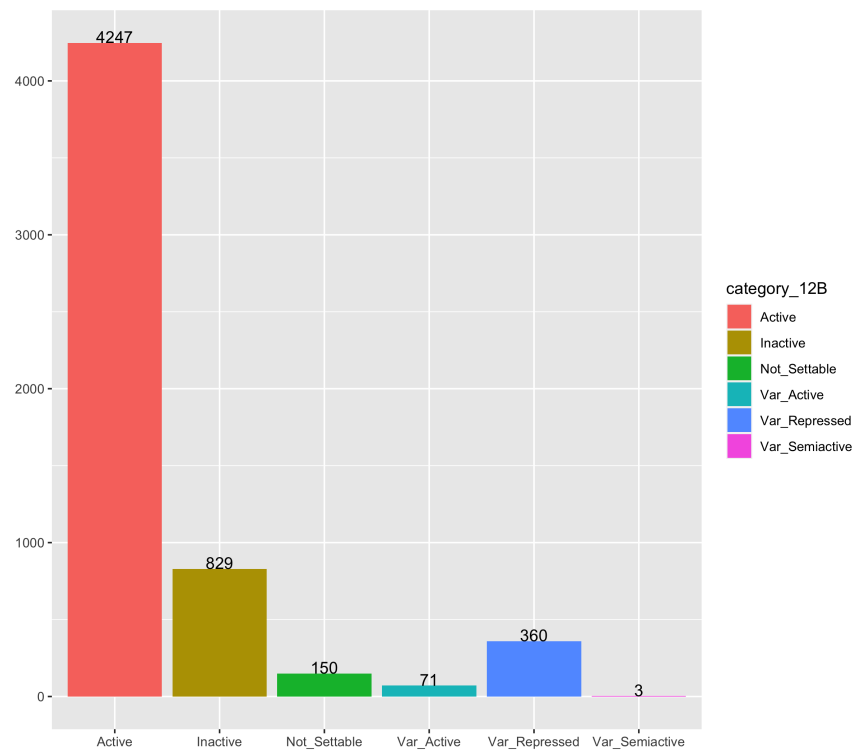
And this are the Clag genes

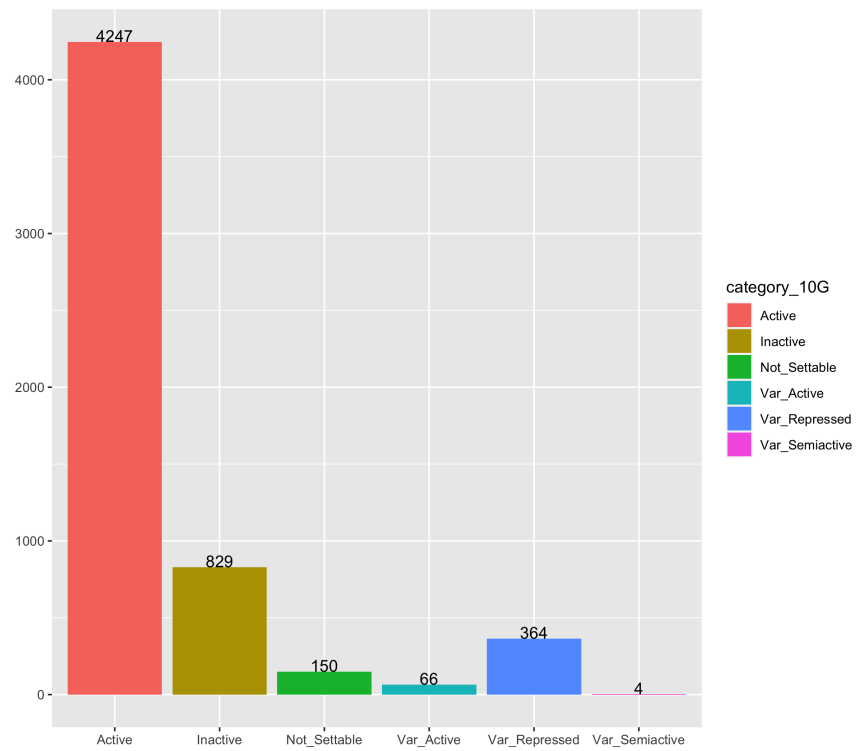
	Gene_id	Variant	Percent_12B	Percent_10G	Percent_3D7B	MaxRedPercentDif	
1	PF3D7_0302200	TRUE	78.68602	99.57983	42.32238	57.25745	
2	PF3D7_0302500	TRUE	98.81589	84.39649	98.98778	14.59129	
	MeanRedPercent	MaxRedPercent	Interval	areaFC	area_12B	area_10G	area_3D7B
1	73.52941	99.57983	Right	4.823089	37.32948	88.19577	23.32522
2	94.06672	98.98778	Right	3.452270	100.12065	53.68762	99.58767
	MaxArea	MinArea	MeanPercent	rel_12B	rel_10G	rel_3D7B	Gene_name
1	88.19577	23.32522	76.36804	min	max	min	CLAG3.2
2	100.12065	53.68762	88.72404	max	min	max	CLAG3.1

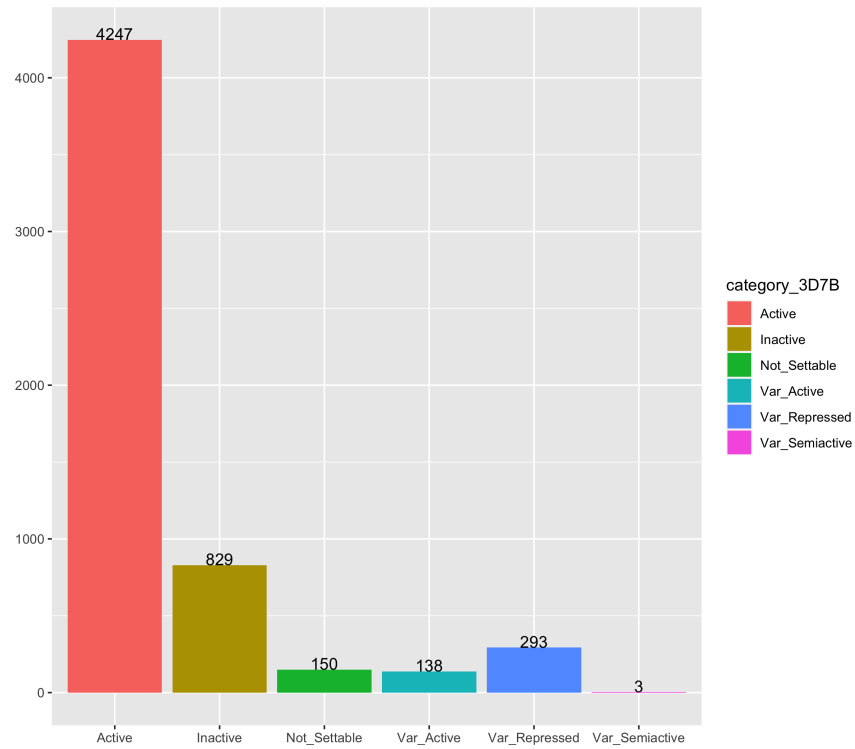
	Annot	state_12B	state_10G
1	cytoadherence linked asexual protein 3.2	Var_Repressed_FC	Var_Active_FC
2	cytoadherence linked asexual protein 3.1	Var_Active_FC	Var_Repressed_FC
	state_3D7B	category_12B	category_10G
1	Var_Repressed_FC	Var_Repressed	Var_Active
2	Var_Active_FC	Var_Active	Var_Repressed

1.7 Category Histograms

For each strain, we plot a histogram of genes in each category.



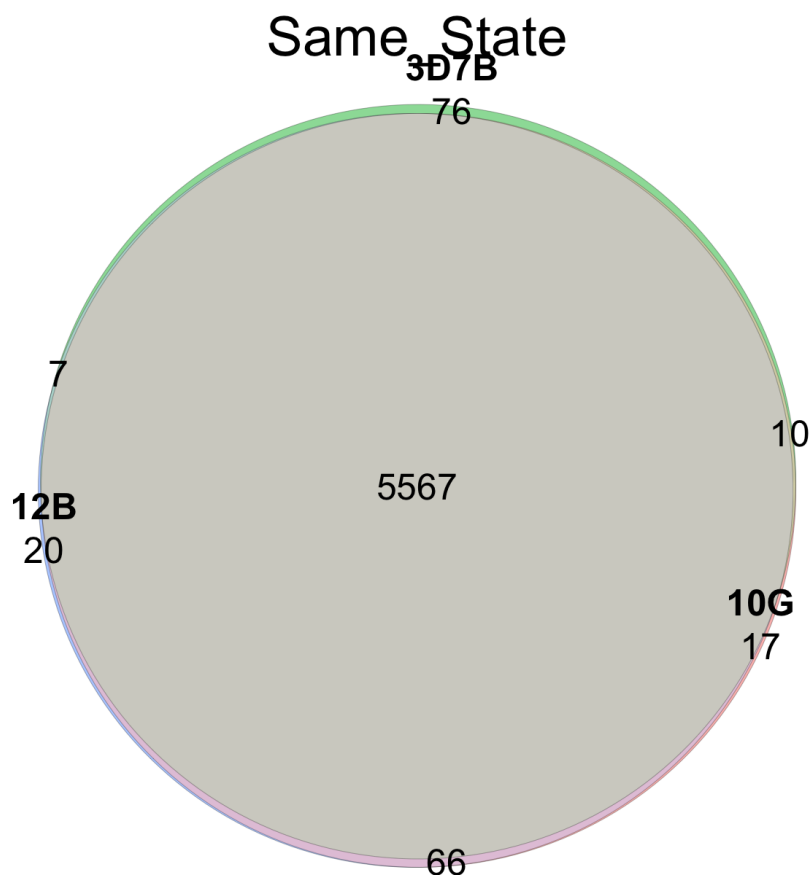




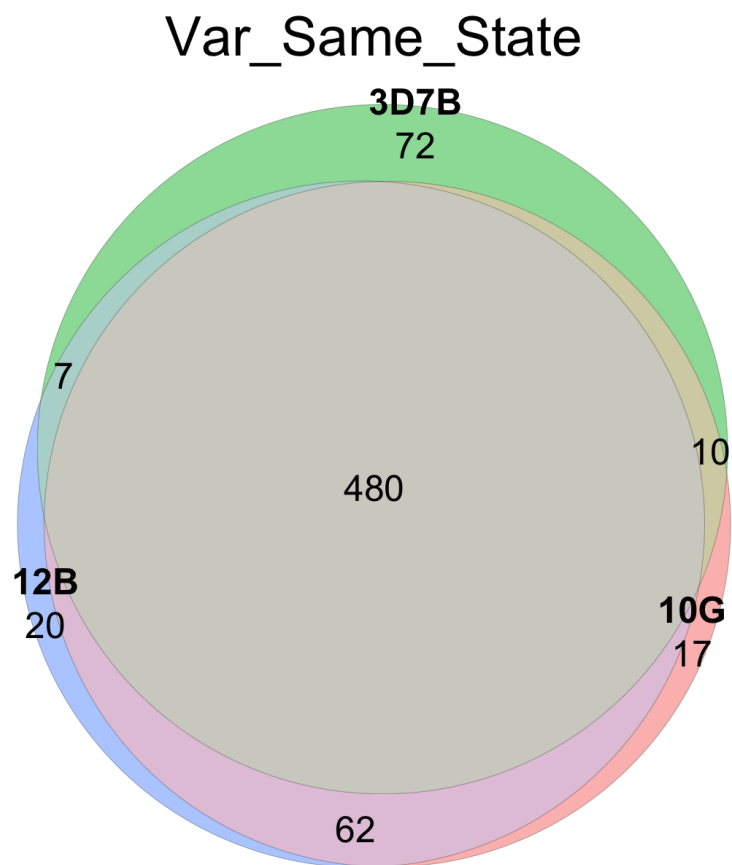
1.8 Venn Diagrams

We now plot Venn diagrams to visualize how the strains compare.

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

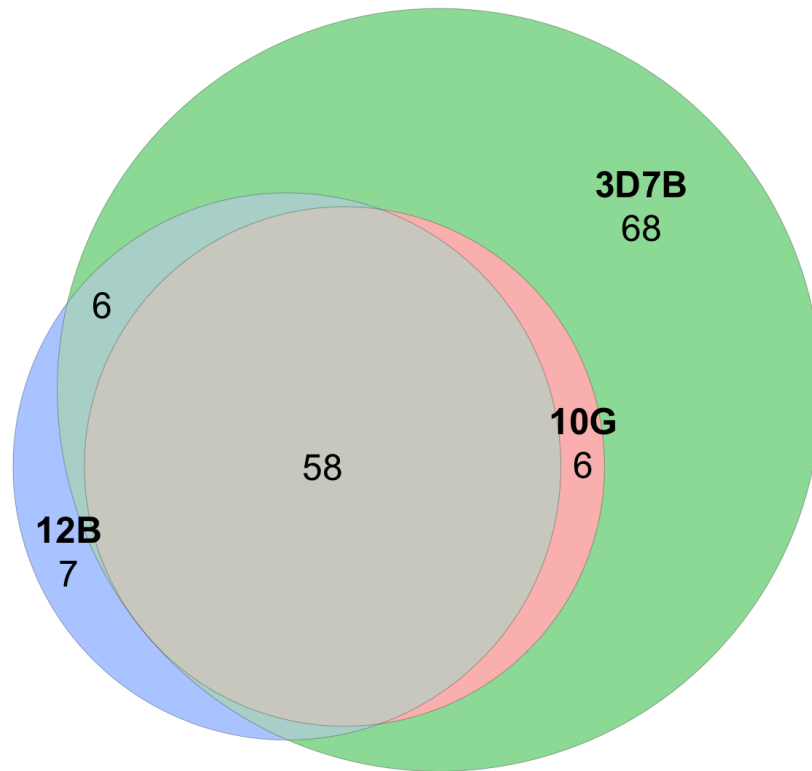


And we do the same but only for clonally variant genes:

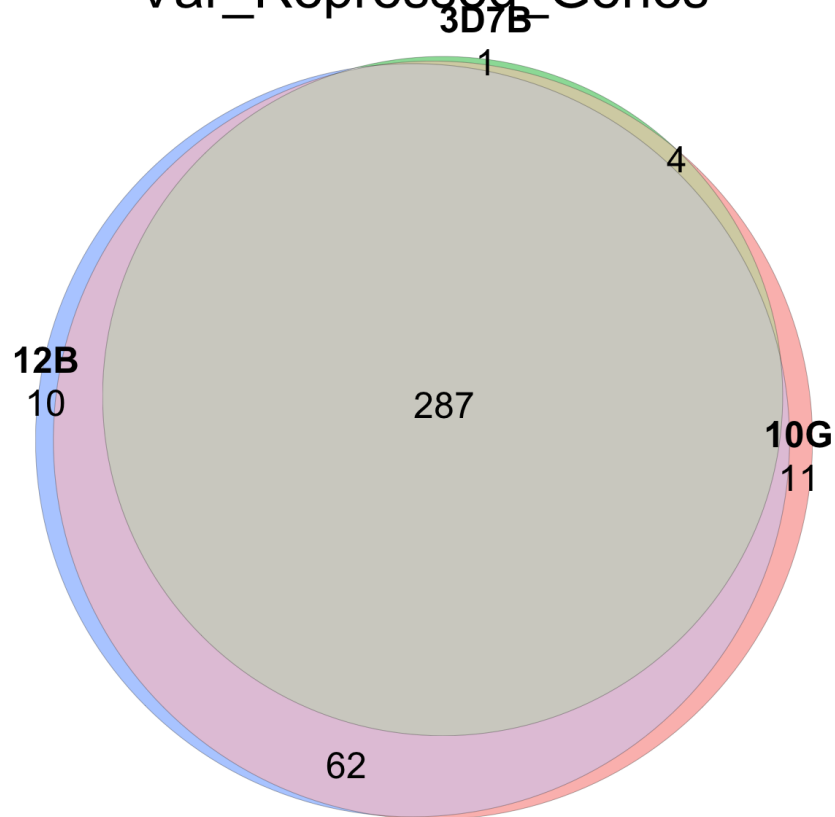


Then we make venn diagrams for the "Var_active" and "Var_Repressed" states.

Var_Active_Genes



Var_Repressed_Genes



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 the
```

```
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)) %>%
  mutate(MaxRedPercent = apply(select(., contains('Percent_')), 1, max))

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 appropriate 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))

```

```
print(area_df, width = 400)
table(duplicated(area_df$Gene_id))
```

2.4 Load RNA-Seq Data

```
## ##### Old approach #####
## 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_pcmt = (rank(Max)/length(Max))*100)

otto <- otto %>% select(Gene_id, Otto_Max_pcmt)
otto <- otto %>% group_by(Gene_id) %>% summarize_all(list(mean))

table(duplicated(otto$Gene_id))
otto %>% filter(duplicated(otto$Gene_id)) %>% print(width = 400)
otto %>% filter(Gene_id == 'PF3D7_0108400') %>% print(width = 400)

hist(otto$Otto_Max_pcmt)
```

```
## 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_pcmt = (rank(Max)/length(Max))*100)
```

```
hoeij <- hoeij %>% select(Gene_id, Hoeij_Max_pcmt)  
hoeij <- hoeij %>% group_by(Gene_id) %>% summarize_all(list(mean))  
hoeij
```

```
hist(hoeij$Hoeij_Max_pcmt)
```

```
## 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_pcmt = (rank(Max)/length(Max))*100)
```

```
toen <- toen %>% select(Gene_id, Toen_Max_pcmt)  
toen <- toen %>% group_by(Gene_id) %>% summarize_all(list(mean))
```

```
toen
```

```
hist(toen$Toen_Max_pcmt)
```

```
## 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 <- bart %>% group_by(Gene_id) %>% summarize_all(list(mean))

bart

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)
table(duplicated(rna_df$Gene_id))

**

```

2.5 Load Annotation

```

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

```

```
distinct() # remove duplicated rows
```

```
print(annot_df, width=200)
table(duplicated(annot_df$Gene_id))
```

2.6 Create Join DF

```
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')
```

```
## Add Variant 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 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 :=
```

```
## 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 <- enquos(outcol)
  areacol <- enquos(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_reddown <- 15
th_areaFC <- 1
th_redpcntdif <- 30

## 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){

```

```

statecol <- enquos(statecol)
redcol <- enquos(redcol)
relcol <- enquos(relcol)

df <- df %>%
  mutate(!! statecol := case_when(

    ## No variant amb FC i RedPcntDif
    ## RNA-Seq & MeanRed
    !Variant &
    areaFC > th_areaFC &
    MeanPercent >= th_rnapcnt &
    MaxRedPercent >= th_reddown &
    MaxRedPercentDif >= th_redpcntdif &
    !! relcol == 'max' ~ 'Active_FC_Dif_max',

    !Variant &
    areaFC > th_areaFC &
    MeanPercent >= th_rnapcnt &
    MaxRedPercent >= th_reddown &
    MaxRedPercentDif >= th_redpcntdif &
    !! relcol == 'mid' ~ 'Active_FC_Dif_mid',

    !Variant &
    areaFC > th_areaFC &
    MeanPercent >= th_rnapcnt &
    MaxRedPercent >= th_reddown &
    MaxRedPercentDif >= th_redpcntdif &
    !! relcol == 'min' ~ 'Inactive_FC_Dif_min',

    ## No variant amb FC no RedPcntDif
    !Variant &
    areaFC > th_areaFC &
    MeanPercent >= th_rnapcnt &
    MaxRedPercent >= th_reddown &
    MaxRedPercentDif < th_redpcntdif ~ 'Active_FC',

    ## No variant amb FC < reddown
    !Variant &
    areaFC > th_areaFC &

```

```

MeanPercent >= th_rnapcnt &
MaxRedPercent < th_reddown ~ 'Inactive_FC_reddown',

## NO-RNA-Seq Rescued
!Variant &
areaFC > th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent >= th_redrescue &
MaxRedPercentDif >= th_redpcntdif &
!! relcol == 'max' ~ 'Active_FC_Dif_rescued_max',

!Variant &
areaFC > th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent >= th_redrescue &
MaxRedPercentDif >= th_redpcntdif &
!! relcol == 'mid' ~ 'Active_FC_Dif_rescued_mid',

!Variant &
areaFC > th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent >= th_redrescue &
MaxRedPercentDif >= th_redpcntdif &
!! relcol == 'min' ~ 'Inactive_FC_Dif_rescued_min',

## FC NO RNA-Seq rescued, no redpcnt dif
!Variant &
areaFC > th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent >= th_redrescue &
MaxRedPercentDif < th_redpcntdif ~ 'Active_FC_rescued',

## FC No RNA-Seq no rescue
!Variant &
areaFC > th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent < th_redrescue ~ 'Inactive_FC',

## No Var, no FC
!Variant &

```

```

areaFC < th_areaFC &
MeanPercent >= th_rnapcnt &
MaxRedPercent >= th_reddown ~ 'Active',

!Variant &
areaFC < th_areaFC &
MeanPercent >= th_rnapcnt &
MaxRedPercent < th_reddown ~ 'Inactive_reddown',

!Variant &
areaFC < th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent > th_redrescue ~ 'Active_rescued',

!Variant &
areaFC < th_areaFC &
MeanPercent < th_rnapcnt &
MaxRedPercent < th_redrescue ~ 'Inactive',

## Var

Variant &
areaFC >= th_areaFC &
MaxRedPercent >= th_reddown &
!! relcol == 'max' ~ 'Var_Active_FC',

Variant &
areaFC >= th_areaFC &
MaxRedPercent >= th_reddown &
!! relcol == 'mid' ~ 'Var_Semiactive_FC',

Variant &
areaFC >= th_areaFC &
MaxRedPercent >= th_reddown &
!! relcol == 'min' ~ 'Var_Repressed_FC',

Variant &
areaFC >= th_areaFC &
MaxRedPercent < th_reddown ~ 'Var_Repressed_FC_reddown',

```

```

Variant &
areaFC < th_areaFC &
MeanRedPercent >= th_redpcnt ~ 'Var_Active_noFC',

Variant &
areaFC < th_areaFC &
MeanRedPercent < th_redpcnt ~ 'Var_Repressed_noFC',

##### Not settable
## No Var, FC NA
!Variant &
is.na(areaFC) &
MeanPercent >= th_rnapcnt &
MaxRedPercent >= th_reddown ~ 'Active_FCna',

!Variant &
is.na(areaFC) &
MeanPercent >= th_rnapcnt &
MaxRedPercent < th_reddown ~ 'Not_Settable',

!Variant &
is.na(areaFC) &
MeanPercent >= th_rnapcnt &
is.na(MaxRedPercent) ~ 'Active_FCna_Redna',

!Variant &
is.na(areaFC) &
MeanPercent < th_rnapcnt &
MaxRedPercent >= th_redrescue ~ 'Not_Settable',

!Variant &
is.na(areaFC) &
MeanPercent < th_rnapcnt &
MaxRedPercent < th_redrescue ~ 'Inactive_FCna',

!Variant &
is.na(areaFC) &
MeanPercent < th_rnapcnt &
is.na(MaxRedPercent) ~ 'Inactive_FCna_Redna',

```

```
## RNA-Seq NA
```

```
!Variant &  
is.na(MeanPercent) &  
areaFC >= th_areaFC &  
MaxRedPercent >= th_redrescue &  
!! relcol == 'max' ~ 'Active_RNASeqNA_rescued_FC_max',
```

```
!Variant &  
is.na(MeanPercent) &  
areaFC >= th_areaFC &  
MaxRedPercent >= th_redrescue &  
!! relcol == 'mid' ~ 'Active_RNASeqNA_rescued_FC_mid',
```

```
!Variant &  
is.na(MeanPercent) &  
areaFC >= th_areaFC &  
MaxRedPercent >= th_redrescue &  
!! relcol == 'min' ~ 'Inactive_RNASeqNA_rescued_FC_min',
```

```
!Variant &  
is.na(MeanPercent) &  
areaFC >= th_areaFC &  
MaxRedPercent < th_redrescue &  
MaxRedPercent > th_reddown ~ 'Not_Settable',
```

```
!Variant &  
is.na(MeanPercent) &  
areaFC >= th_areaFC &  
MaxRedPercent < th_redrescue &  
MaxRedPercent >= th_reddown ~ 'Not_Settable',
```

```
!Variant &  
is.na(MeanPercent) &  
areaFC >= th_areaFC &  
MaxRedPercent < th_reddown ~ 'Inactive_FC_reddown',
```

```
!Variant &  
is.na(MeanPercent) &
```



```

areaFC >= th_areaFC &
is.na(MaxRedPercent) ~ 'Not_Settable',

!Variant &
is.na(MeanPercent) &
areaFC < th_areaFC &
MaxRedPercent >= th_redrescue ~ 'Active_RNASeqNA_rescued',

!Variant &
is.na(MeanPercent) &
areaFC < th_areaFC &
MaxRedPercent < th_redrescue &
MaxRedPercent >= th_reddown ~ 'Not_Settable',

!Variant &
is.na(MeanPercent) &
areaFC < th_areaFC &
MaxRedPercent < th_reddown ~ 'Inactive_RNASeqNA_reddown',

!Variant &
is.na(MeanPercent) &
areaFC < th_areaFC &
is.na(MaxRedPercent) ~ 'Not_Settable',

## VARs
Variant &
is.na(areaFC) &
MeanRedPercent >= th_redpcnt ~ 'Var_Active_FCna',

Variant &
is.na(areaFC) &
MeanRedPercent < th_redpcnt ~ 'Var_Repressed_FCna',

Variant &
is.na(areaFC) &
is.na(MeanRedPercent) ~ 'Not_Settable',

## Double NAs
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 <- enquos(statecol)
  categorycol <- enquos(categorycol)

  df <- df %>%
    mutate(!! categorycol := case_when(
      startsWith(!! statecol, 'Active') ~ 'Active',
      startsWith(!! statecol, 'Inactive') ~ 'Inactive',
      startsWith(!! statecol, 'Var_Active') ~ 'Var_Active',
      startsWith(!! statecol, 'Var_Semiactive') ~ 'Var_Semiactive',
      startsWith(!! statecol, 'Var_Repressed') ~ 'Var_Repressed',
      startsWith(!! statecol, 'Not_Settable') ~ 'Not_Settable',
      TRUE ~ 'No_Category'))

  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) %>%
  set_category(state_12B, category_12B) %>%
  set_category(state_10G, category_10G) %>%
  set_category(state_3D7B, category_3D7B)

print(state_df, width = 400)

table(state_df$state_3D7B)

```

```
state_df %>% filter(category_12B == 'No_Category!') %>% print(width = 400)
```

2.8 Some checks and results

```
## We check no rows are set to "Wrong!"
```

```
state_df %>%
  filter(state_12B == 'Not_Settable' |
         state_10G == 'Not_Settable' |
         state_3D7B == 'Not_Settable') %>%
  print(width = 400)
```

```
state_df %>%
  filter(category_12B == 'No_Category' |
         category_10G == 'No_Category' |
         category_3D7B == 'No_Category') %>%
  print(width = 400)
```

```
## Save results
```

```
write.csv(state_df, './Results_Tables/gene_state_final.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 Histograms of classification

```
## Categories histograms
```

```
make_histogram <- function(df, column){
  col <- enquo(column)

  df %>%
    select(!!col) %>%
    count(!!col) %>%
    ggplot(aes(y=n, x=!!col, fill=!!col)) +
    geom_bar(stat='identity') +
    geom_text(aes(label=n), vjust=0) +
    theme(
      plot.title = element_blank(),
      axis.title.x = element_blank(),
      axis.title.y = element_blank()
    )
}

hist12B <- make_histogram(state_df, category_12B)
hist10G <- make_histogram(state_df, category_10G)
hist3D7B <- make_histogram(state_df, category_3D7B)

ggsave(hist12B, filename = './Plots/histogram_12B.png', height=7, width=8)
ggsave(hist10G, filename = './Plots/histogram_10G.png', height=7, width=8)
ggsave(hist3D7B, filename = './Plots/histogram_3D7B.png', height=7, width=8)
```

2.10 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')

```

2.11 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)

varstate <- state_df %>%
  filter(Variant) %>%
  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(varstate$gs_12B, varstate$gs_10G, varstate$gs_3D7B, 'Var_Same_State')

```