QTL-Sorghum

Michael Hall

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```
devtools::install github("PBGLMichaelHall/QTLseqr", force = TRUE)
Downloading GitHub repo PBGLMichaelHall/QTLseqr@HEAD
* checking for file '/tmp/RtmppmDOD6/remotes2f256e86d541/PBGLMichaelHall-QTLseqr-856f201/DESCRIPTION' .
* preparing 'QTLseqr':
* checking DESCRIPTION meta-information ... OK
* cleaning src
Warning: /tmp/Rtmp00kH7r/Rbuild2f67649838af/QTLseqr/man/tricube_Smooth.Rd:2: unexpected '}'
Warning: /tmp/Rtmp00kH7r/Rbuild2f67649838af/QTLseqr/man/tricube_Smooth.Rd:3: unexpected '}'
* checking for LF line-endings in source and make files and shell scripts
* checking for empty or unneeded directories
Omitted 'LazyData' from DESCRIPTION
* building 'QTLseqr_0.7.5.2.tar.gz'
Installing package into '/home/michael/R/x86_64-pc-linux-gnu-library/4.1'
(as 'lib' is unspecified)
library(QTLseqr)
library(tinytex)
library(vcfR)
   ****
                   vcfR
               ***
   This is vcfR 1.12.0
    browseVignettes('vcfR') # Documentation
     citation('vcfR') # Citation
   ****
               ****
                          ****
                                      ****
library(tidyr)
library(ggplot2)
library(dplyr)
Attaching package: 'dplyr'
The following objects are masked from 'package:stats':
   filter, lag
The following objects are masked from 'package:base':
    intersect, setdiff, setequal, union
library(ggrepel)
#Set Working Directory
setwd("/home/michael/Desktop/QTLseqr/extdata")
```

```
#vcf file must only contain bialleleic variants. (filter upstream, e.g., with bcftools view -m2 -M2), a
vcf <- read.vcfR(file = "freebayes_D2.filtered.vcf")</pre>
Scanning file to determine attributes.
File attributes:
 meta lines: 937
 header_line: 938
 variant count: 7861
 column count: 13
Meta line 937 read in.
All meta lines processed.
gt matrix initialized.
Character matrix gt created.
 Character matrix gt rows: 7861
 Character matrix gt cols: 13
 skip: 0
 nrows: 7861
 row_num: 0
Processed variant 1000Processed variant 2000Processed variant 3000Processed variant 4000Processed varia
All variants processed
#Convert to tidy data frame
VCF_TIDY <- vcfR2tidy(vcf)</pre>
#Call the Parser
QTLParser_1_MH(vcf = VCF_TIDY, HighBulk = "D2_F2_tt", LowBulk = "D2_F2_TT")
'data.frame': 31424 obs. of 7 variables:
$ CHROM : int 1 1 1 1 1 1 1 1 1 1 ...
$ POS
       : int 344698 2943267 3751995 4720049 5567202 6237654 6582529 7047748 8720466 8720551 ...
$ REF
        : chr "C" "T" "T" "G" ...
$ ALT : chr "T" "A" "C" "A" ...
        : int 6 30 8 30 22 10 33 1 3 1 ...
$ var1 : chr "14,23" "66,51" "15,10" "80,37" ...
$ Samples: chr "con-all" "con-all" "con-all" "...
'data.frame': 31400 obs. of 7 variables:
$ CHROM : int 1 1 1 1 1 1 1 1 1 ...
       : int 344698 2943267 3751995 4720049 5567202 6237654 6582529 7047748 8720466 8720551 ...
$ REF
         : chr "C" "T" "T" "G" ...
$ ALT : chr "T" "A" "C" "A" ...
$ DP
        : int 6 30 8 30 22 10 33 1 3 1 ...
$ var1 : chr "19,18" "44,42" "8,4" "64,50" ...
$ Samples: chr "con-all" "con-all" "con-all" "...
#Set High bulk and Low bulk sample names and parser generated file name
HighBulk <- "D2_F2_tt"</pre>
LowBulk <- "D2_F2_TT"
file <- "Hall.csv"</pre>
#Choose which chromosomes will be included in the analysis,
#the tidy data frame makes a CHROMKEY so no need to change chromosome names
Chroms <- 1:10
```

```
df <-
importFromTable(
    file = file,
    highBulk = HighBulk,
    lowBulk = LowBulk,
    chromList = Chroms
)

#plot histograms associated with filtering arguments to determine if cut off values are appropriate

ggplot(data = df) +
    geom_histogram(aes(x = AD_ALT.LOW + AD_ALT.HIGH)) + xlim(0,400)</pre>
```

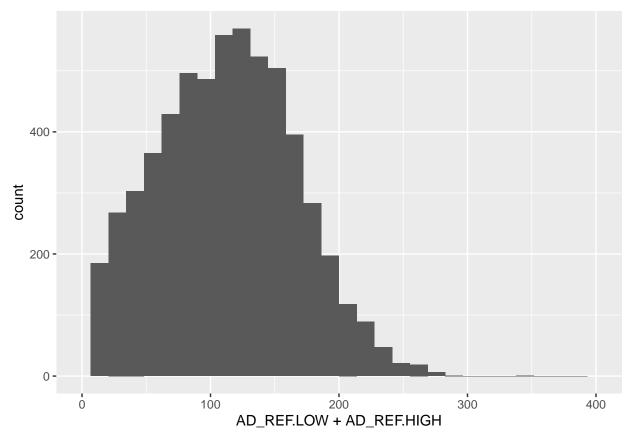
```
0 100 200 300 400

AD_ALT.LOW + AD_ALT.HIGH

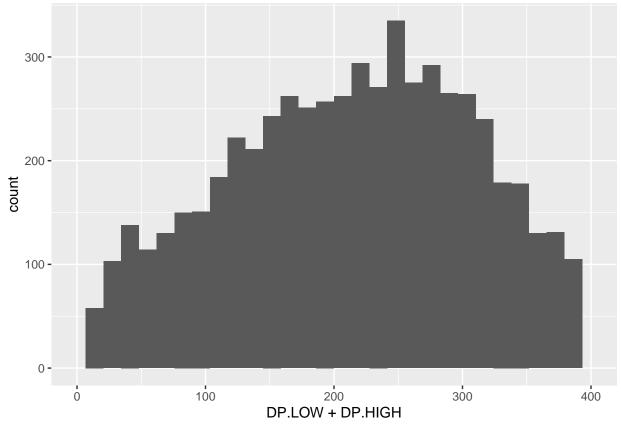
ggsave(filename = "AD_Histogram.png",plot = last_plot())
ggplot(data = df) +
geom_histogram(aes(x = AD_REF.LOW + AD_REF.HIGH)) + xlim(0,400)
```

200 -

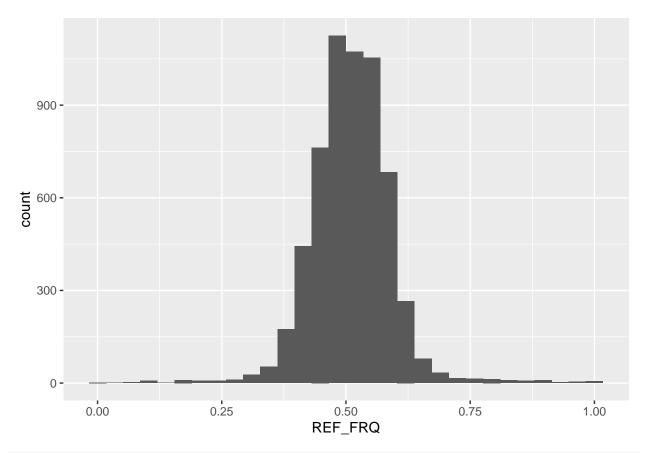
0 -



```
ggsave(filename = "AD_Ref_Histogram.png",plot = last_plot())
ggplot(data =df) +
  geom_histogram(aes(x = DP.LOW + DP.HIGH)) + xlim(0,400)
```



```
ggsave(filename = "Depth_Histogram.png",plot=last_plot())
ggplot(data = df) +
  geom_histogram(aes(x = REF_FRQ))
```



```
ggsave(filename = "Ref_Freq_Histogram.png",plot = last_plot())

#Filter SNPs based on some criteria

df_filt <-
    filterSNPs(
        SNPset = df,
        refAlleleFreq = 0.20,
        minTotalDepth = 100,
        maxTotalDepth = 400,
        minSampleDepth = 40,
        # minGQ = 0
)</pre>
```

...Filtered 86 SNPs

Filtering by total sample read depth: Total DP >= 100
...Filtered 733 SNPs

Filtering by total sample read depth: Total DP <= 400
...Filtered 175 SNPs

Filtering by per sample read depth: DP >= 40
...Filtered 22 SNPs

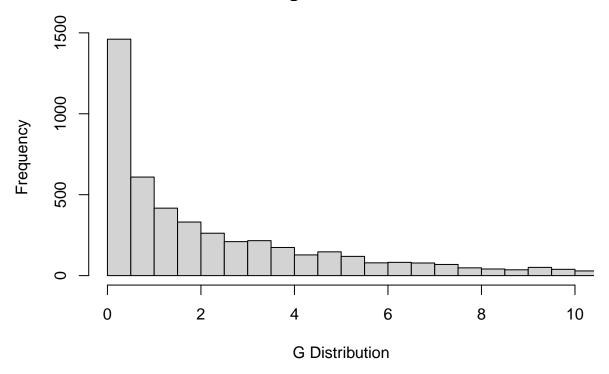
Filtering by reference allele frequency: 0.2 <= REF_FRQ <= 0.8

Original SNP number: 5917, Filtered: 1016, Remaining: 4901

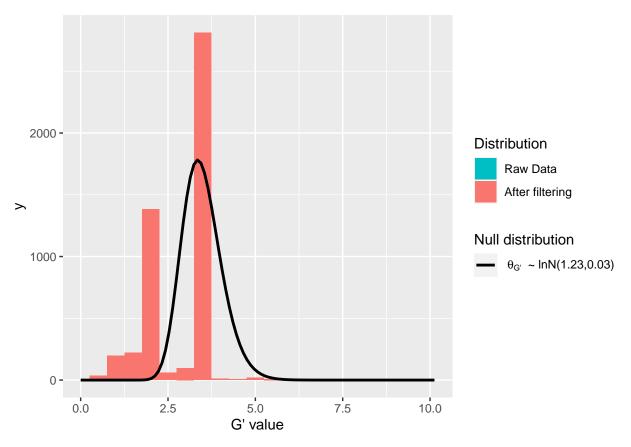
```
#Run G' analysis
df_filt<-runGprimeAnalysis_MH(</pre>
 SNPset = df_filt,
 windowSize = 5000000,
 outlierFilter = "deltaSNP",
filterThreshold = 0.1)
Counting SNPs in each window...
Calculating tricube smoothed delta SNP index...
Calculating G and G' statistics...
Using deltaSNP-index to filter outlier regions with a threshold of 0.1
Estimating the mode of a trimmed G prime set using the 'modeest' package...
Calculating p-values...
#Run QTLseq analysis
df_filt2 <- runQTLseqAnalysis_MH(</pre>
 SNPset = df_filt,
 windowSize = 5000000,
 popStruc = "F2",
 bulkSize = c(45, 38),
 replications = 10000,
 intervals = c(95, 99)
Counting SNPs in each window...
Calculating tricube smoothed delta SNP index...
Returning the following two sided confidence intervals: 95, 99
Variable 'depth' not defined, using min and max depth from data: 40-198
Assuming bulks selected from F2 population, with 45 and 38 individuals per bulk.
Simulating 10000 SNPs with reads at each depth: 40-198
Keeping SNPs with >= 0.3 SNP-index in both simulated bulks
Joining, by = "tricubeDP"
setwd("/home/michael/Desktop/SorghumQTL/GPrimeDistributionPlots/")
#Plot G Statistic Distribution
```

hist(df_filt2\$G,breaks = 950,xlim = c(0,10),xlab = "G Distribution",main = "Histogram of G Values")

Histogram of G Values



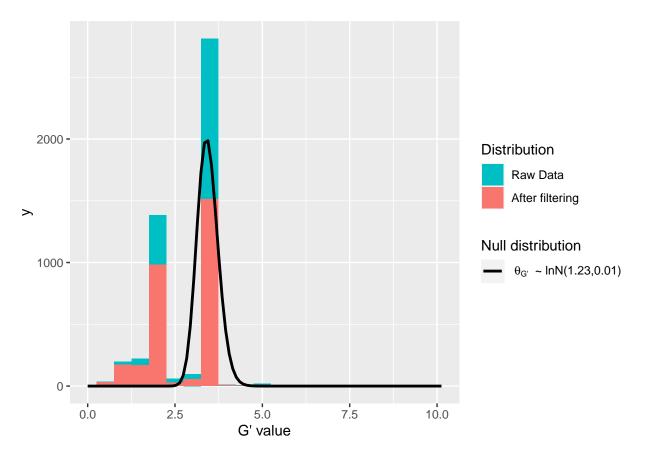
```
# G' Distribution Plot
plotGprimeDist_MH(SNPset = df_filt2, outlierFilter = "Hampel")
```



```
ggsave(filename = "Hampel_GPrime.png",plot = last_plot())
```

Saving 6.5 x 4.5 in image

```
setwd("/home/michael/Desktop/SorghumQTL/DeltaSNP/")
plotGprimeDist_MH(SNPset = df_filt2, outlierFilter = "deltaSNP",filterThreshold = 0.1)
```



```
ggsave(filename = "DeltaSNP.png",plot = last_plot())
```

```
Saving 6.5 x 4.5 in image
```

```
setwd("/home/michael/Desktop/SorghumQTL/nSNPs/")

#make the Plot
snpnumber <- plotQTLStats(SNPset = df_filt2, var = "nSNPs")
ggsave(filename = "nSNPs.png",plot = last_plot())</pre>
```

Saving 6.5 x 4.5 in image

```
setwd("/home/michael/Desktop/SorghumQTL/GPrimeDistributionPlots/")
Gprime<-plotQTLStats(SNPset = df_filt, var = "Gprime", plotThreshold = TRUE, q = 0.01)
ggsave(filename = "GPrime.png",plot = last_plot())</pre>
```

Saving 6.5×4.5 in image

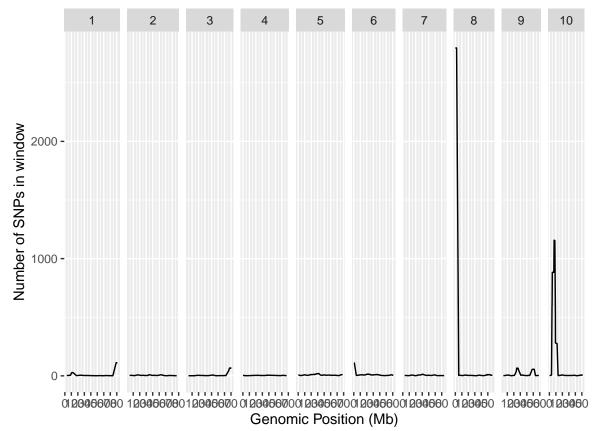
```
setwd("/home/michael/Desktop/SorghumQTL/DeltaSNP/")
deltaSNP<-plotQTLStats(SNPset = df_filt2, var = "deltaSNP", plotIntervals = TRUE)
ggsave(filename = "DeltaSNPInterval.png",plot = last_plot())</pre>
```

Saving 6.5×4.5 in image

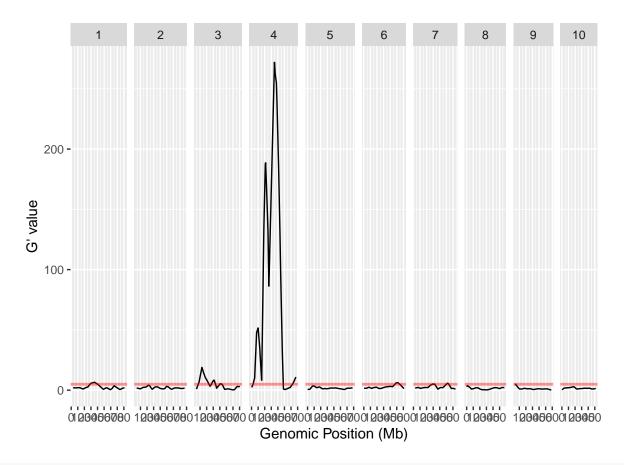
```
setwd("/home/michael/Desktop/SorghumQTL/negLog10Pval/")
neglog<-plotQTLStats(SNPset = df_filt2, var = "negLog10Pval",plotThreshold = TRUE,q=0.01,subset = c("1"
ggsave(filename = "negLog10Pval.png",plot = last_plot())</pre>
```

Saving 6.5×4.5 in image

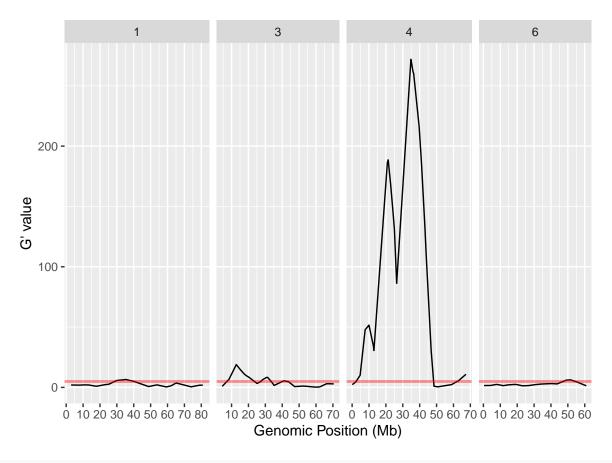




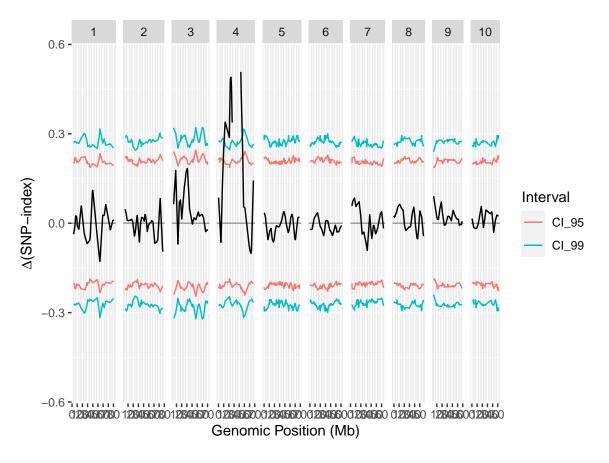
 ${\tt Gprime}$



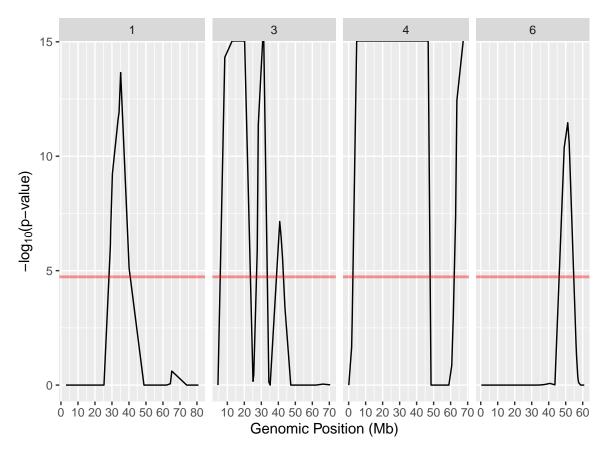
Gprime2



deltaSNP

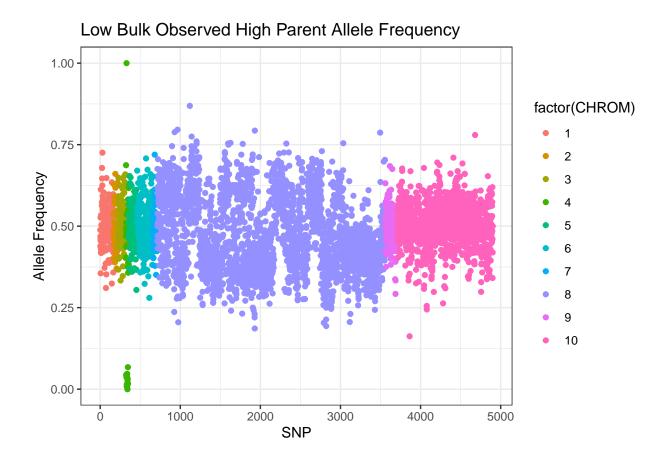


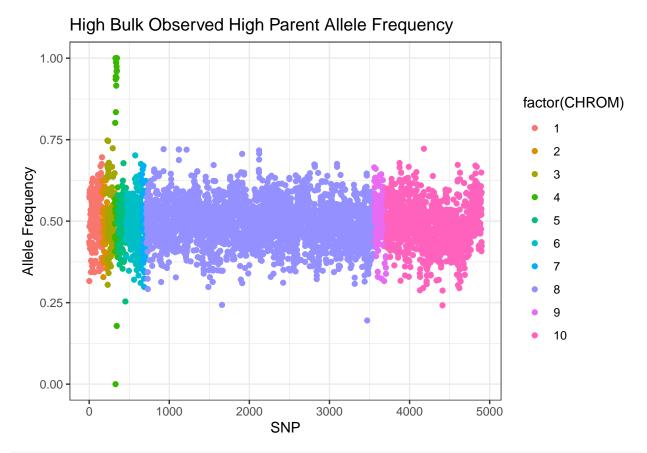
neglog



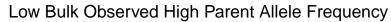
```
#export summary CSV
setwd("/home/michael/Desktop/SorghumQTL/PeakSummary/")
QTLTable <- getQTLTable(SNPset = df_filt, alpha = 0.01, export = TRUE, fileName = "my_BSA_QTL.csv")
write.csv(QTLTable, file = "QTLTablePeaks.csv", row.names = FALSE, col.names = TRUE)
Table4 <- read.table(file = "QTLTablePeaks.csv",header = TRUE, sep = ",", fill=TRUE)

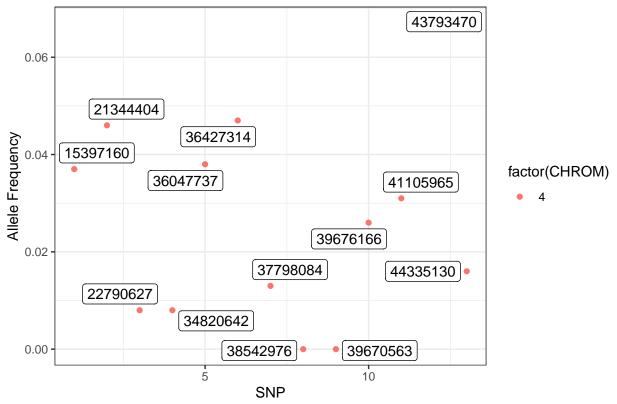
#Use the function to plot allele frequencies per chromosome
Obs_Allele_Freq(SNPSet = df_filt)</pre>
```

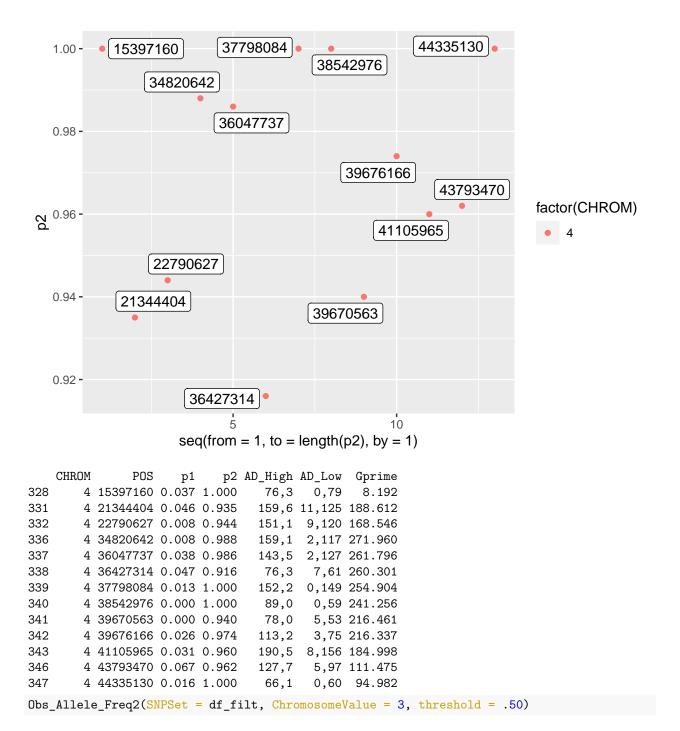




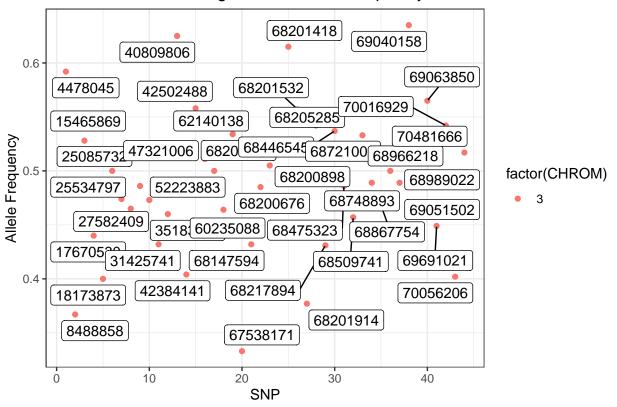
##Use the function to investigate chromosomal region of interest
Obs_Allele_Freq2(SNPSet = df_filt, ChromosomeValue = 4, threshold = .90)

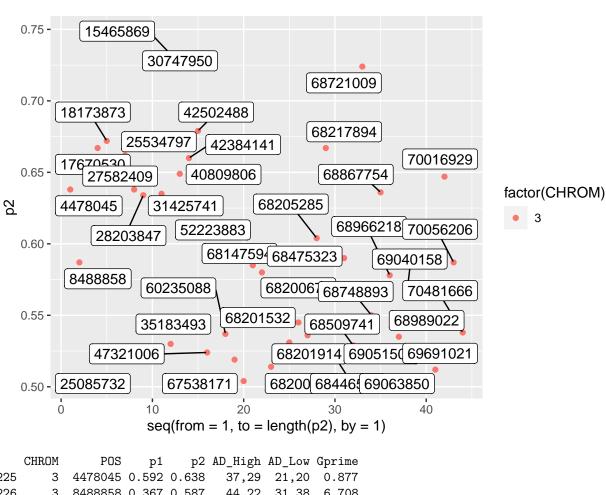






Low Bulk Observed High Parent Allele Frequency

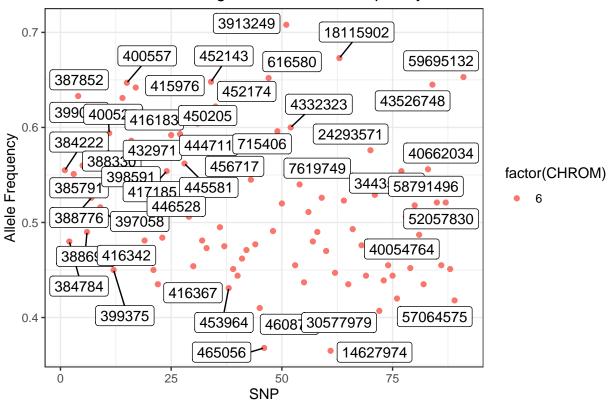


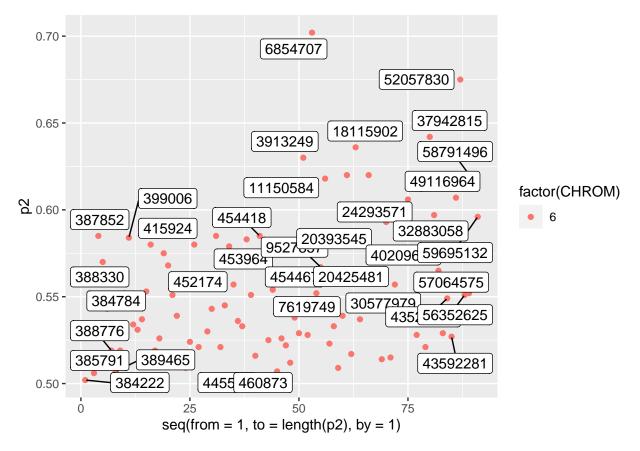


```
225
226
           8488858 0.367 0.587
                                  44,22
                                        31,38 6.708
                                142,66
                                        48,59 14.266
228
        3 15465869 0.528 0.747
229
                                        19,28 10.890
        3 17670530 0.440 0.667
                                  38,22
230
        3 18173873 0.400 0.672
                                  78,34
                                        38,51 10.332
233
        3 25085732 0.500 0.506
                                  40,36
                                        39,36 3.282
234
        3 25534797 0.474 0.662
                                  43,27
                                        22,30
                                               3.536
235
                                        38,38 5.150
        3 27582409 0.465 0.638
                                  67,33
236
        3 28203847 0.486 0.634
                                  85,52
                                        49,55
                                                6.197
237
        3 30747950 0.473 0.746
                                  44,35
                                        15,39
                                                8.295
        3 31425741 0.432 0.635
                                        80,71
238
                                139,54
                                                8.241
240
        3 35183493 0.460 0.530
                                 35,29
                                        31,34
                                                1.615
241
        3 40809806 0.625 0.649
                                  61,50
                                        33,30
                                               5.408
245
        3 42384141 0.404 0.660
                                  64,40
                                        33,59
                                                5.097
246
        3 42502488 0.558 0.679
                                  91,43
                                        43,34
                                                5.070
248
        3 47321006 0.511 0.524
                                  33,24
                                        30,23
                                                0.656
249
        3 52223883 0.500 0.598
                                  64,35
                                        43,35
                                               1.085
252
        3 60235088 0.464 0.537
                                  36,26
                                        31,30
                                                0.256
        3 62140138 0.534 0.519
                                        38,27
                                                0.378
253
                                  41,31
255
        3 67538171 0.333 0.504
                                        61,76
                                                3.018
                                  62,38
259
                                        56,46
        3 68147594 0.432 0.585
                                  79,35
                                               2.998
262
        3 68200676 0.485 0.580
                                  76,48
                                        55,51
                                                2.996
263
        3 68200838 0.505 0.514
                                  55,49
                                        52,48
                                                2.996
264
        3 68200898 0.496 0.507
                                  73,58
                                        71,59
                                                2.996
                                104,99 92,62
279
        3 68201418 0.615 0.531
                                               2.996
```

```
280
        3 68201532 0.557 0.545 104,102 87,81
                                                2.996
284
        3 68201914 0.377 0.536
                                  98,58
                                        85,96
                                                2.996
        3 68205285 0.542 0.604
                                  32,26
                                        21,22
                                                2.996
285
286
        3 68217894 0.431 0.667
                                  62,31
                                        31,41
                                                2.995
289
        3 68446545 0.537 0.521
                                 50,43
                                        46,37
                                                2.988
290
        3 68475323 0.486 0.590
                                105,70
                                        73,74
                                                2.986
292
        3 68509741 0.457 0.529
                                  55,32
                                        49.38
                                                2.985
                                        21,28
294
        3 68721009 0.533 0.724
                                  55,32
                                                2.977
296
        3 68748893 0.489 0.550
                                 71,44
                                        58,46
                                                2.976
298
        3 68867754 0.468 0.636
                                  56,37
                                        32,42
                                                2.971
301
        3 68966218 0.500 0.578
                                  48,32
                                        35,32
                                                2.968
302
                                        80,67
        3 68989022 0.489 0.535
                                  92,64
                                                2.967
307
        3 69040158 0.635 0.572
                                 83,80
                                        62,46
                                                2.965
                                        61,56
308
        3 69051502 0.462 0.527
                                  68,48
                                                2.964
310
        3 69063850 0.565 0.507
                                 70,78
                                        68,60
                                                2.964
314
        3 69691021 0.449 0.512
                                103,75
                                        98,92
                                                2.940
317
        3 70016929 0.542 0.647
                                  44,26
                                        24,22
                                                2.928
318
        3 70056206 0.402 0.587
                                  71,43
                                        50,64
                                               2.926
319
        3 70481666 0.517 0.538
                                 85,78 73,73 2.910
Obs_Allele_Freq2(SNPSet = df_filt, ChromosomeValue = 6, threshold = .50)
```

Low Bulk Observed High Parent Allele Frequency



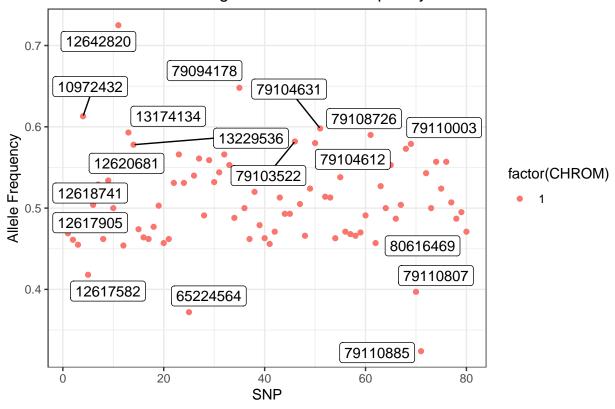


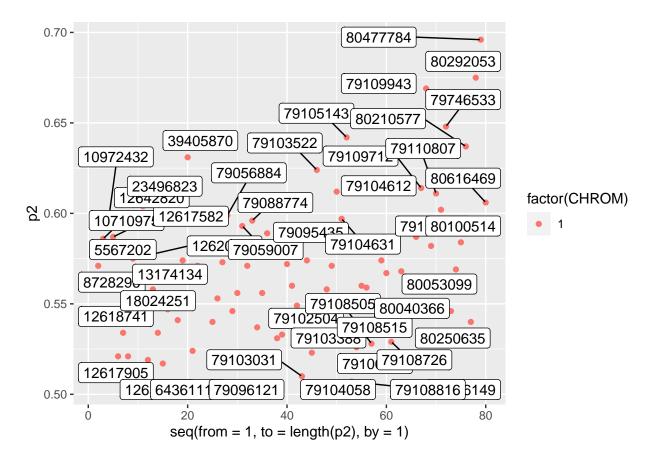
```
CHROM
               POS
                             p2 AD_High AD_Low Gprime
                       р1
                                  103,76 102,61
469
        6
            384222 0.555 0.502
                                                  1.539
473
        6
            384784 0.480 0.552
                                   96,73 78,79
                                                  1.539
            385791 0.551 0.506
                                          83,66
474
        6
                                   85,81
                                                  1.539
475
        6
            387852 0.633 0.585 103,105
                                          73,61
                                                  1.539
476
        6
            388330 0.560 0.570
                                   69,51
                                          52,40
                                                  1.539
477
            388697 0.490 0.543
                                  107,77
                                          90,80
                                                  1.539
478
        6
            388776 0.526 0.519
                                   56,40
                                          52,36
                                                  1.539
480
        6
            389465 0.506 0.507
                                   69,43
                                          67,42
                                                  1.539
483
        6
            397058 0.516 0.519
                                   69,65
                                          64,61
                                                  1.539
                                          39,21
487
        6
            398591 0.580 0.552
                                   48,29
                                                  1.539
            399006 0.594 0.584
                                          37,28
488
        6
                                   52,41
                                                  1.539
493
        6
            399375 0.450 0.534
                                   62,45
                                          54,55
                                                  1.539
495
        6
            399740 0.494 0.531
                                   76,84
                                          67,86
                                                  1.539
498
        6
            400527 0.631 0.537
                                   36,41
                                          31,24
                                                  1.539
                                          34,24
499
            400557 0.647 0.553
                                   42,44
        6
                                                  1.539
502
        6
            415924 0.586 0.580
                                   51.41
                                          37,29
                                                  1.540
503
        6
            415976 0.642 0.519
                                   41,43
                                          38,24
                                                  1.540
504
        6
            416183 0.620 0.526
                                   71,44
                                          64,27
                                                  1.540
                                          45,28
505
        6
            416252 0.481 0.575
                                   61,26
                                                  1.540
506
                                          48,27
                                                  1.540
        6
            416291 0.471 0.568
                                   63,24
507
        6
            416342 0.450 0.551
                                   59,27
                                          48,33
                                                  1.540
                                          47,35
508
        6
            416367 0.435 0.539
                                   55,27
                                                  1.540
509
        6
            416386 0.484 0.510
                                   51,30
                                          49,32
                                                  1.540
510
        6
            417185 0.554 0.509
                                   59,62
                                          57,50
                                                  1.540
        6
                                   66,74 60,51
515
            432057 0.592 0.524
                                                  1.540
```

```
80,74 58,56 1.540
516
           432971 0.569 0.580
525
           444711 0.593 0.521
                                73,64
                                       67,44 1.540
       6
527
           445581 0.562 0.504
                                69,68
                                       68,53
                                             1.540
532
                                97,82 86,80 1.540
           446528 0.506 0.530
       6
534
       6
           448103 0.454 0.543
                                95,69
                                       80,83
                                             1.540
536
       6
           450205 0.604 0.585
                                76,55 54,36 1.540
537
           450665 0.481 0.521
                                61,39
                                       56,42 1.540
       6
                                66,43 55,48 1.540
538
           450674 0.473 0.545
       6
543
       6
           452143 0.648 0.579
                                55,59
                                       40,32 1.540
544
           452174 0.622 0.557
                                       39,37
       6
                                49,61
                                             1.540
546
       6
           453167 0.495 0.536
                                45,51
                                      39,52 1.540
547
           453219 0.475 0.533
                                56,48 49,53 1.540
       6
548
           453964 0.431 0.583
                                67,44 48,58 1.540
       6
                                      62,56 1.540
549
           454157 0.451 0.551
                                76,46
550
           454370 0.444 0.516
                                66,36 62,45 1.540
       6
551
       6
           454418 0.462 0.585
                                69,30
                                      49,35
                                             1.540
552
           454467 0.471 0.569
                                62,24 47,27
                                             1.540
       6
553
                                      96,76 1.540
           456717 0.545 0.525
                               106,91
554
           459132 0.477 0.554
                                46,31 37,34 1.540
       6
559
           460873 0.410 0.507
                                77,66 75,95
                                             1.540
561
       6
           465056 0.368 0.526
                                50,28 45,48 1.540
563
           616580 0.652 0.522
                                60,58 55,31 1.542
       6
           639623 0.491 0.512
                               107,79 102,82 1.542
566
       6
567
           715406 0.596 0.538
                                71,68 61,46 1.543
       6
568
           720164 0.520 0.529
                                72,66 64,61 1.543
       6
572
       6
          3913249 0.708 0.630
                                51,46
                                      30,19 1.581
573
       6
          4332323 0.600 0.528
                                28,33
                                       25,22 1.624
575
          6854707 0.455 0.702
                                40,25
                                      17,30 2.239
576
       6 7619749 0.540 0.552
                               116,87
                                      94,74 2.426
580
       6 9527857 0.437 0.567
                                93,52 71,67 2.061
                                      42,46 1.601
581
       6 11150584 0.511 0.618
                                68,48
582
       6 11731523 0.480 0.523
                                46,36 42,39 1.436
584
       6 12004221 0.490 0.533
                                       28,25 1.493
                                32,24
586
       6 13011995 0.526 0.509
                                55,40 53,36 1.714
587
       6 14373081 0.470 0.539
                                76,62
                                       65,70 2.011
588
       6 14627974 0.365 0.620
                                49,19 30,33 2.067
589
       6 14735537 0.447 0.517
                                31,21
                                      29,26 2.091
591
       6 18115902 0.673 0.636
                                49,35
                                       28,17 2.426
592
       6 18118536 0.523 0.537
                                36,34 31,31 2.426
593
       6 18399688 0.435 0.603
                                41,30 27,39 2.444
595
       6 18675107 0.493 0.620
                                93,67 57,69 2.461
598
       6 20393545 0.542 0.574
                               105,90 78,76 2.155
599
       6 20425481 0.476 0.571
                                36,30 27,33 2.145
602
       6 22043951 0.444 0.514
                                76,55 72,69 1.632
609
       6 24293571 0.576 0.593
                                73,72 50,53 1.363
612
       6 25872403 0.529 0.515
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                                       33,24 1.445
620
                                39,22 31,32 2.279
       6 30577979 0.407 0.557
621
       6 32392854 0.439 0.536
                                37,18 32,23 2.525
623
       6 32883058 0.455 0.593
                                54,20
                                      37,24 2.589
624
                                       37,25 2.589
       6 32883065 0.444 0.606
                                57,20
625
       6 34368555 0.420 0.619
                                39,21
                                       24,29 2.785
626
       6 34435846 0.554 0.528
                                       42,33 2.793
                                47,41
627
       6 34770268 0.506 0.612 134,86
                                       85,84 2.815
629
       6 35637255 0.452 0.521
                                38,28 35,34 2.868
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634
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                                83,55 56,58
636
       6 40054764 0.487 0.597
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637
        6 40209616 0.435 0.565
                               104,54
                                       80,70
                                              3.121
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        6 40662034 0.556 0.529
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                                       56,48
                                             3.142
                                79,69
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640
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641
        6 43592281 0.521 0.527
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                                       71,46 2.891
                                       55,79
643
        6 49116964 0.455 0.607
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                                             6.017
       6 52057830 0.521 0.675
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646
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       6 56352625 0.451 0.551
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                                       57,56
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       6 57064575 0.418 0.552
650
                               100,59
                                       81,82
                                              3.514
653
        6 58791496 0.539 0.617
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                                       70,71
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654
        6 59695132 0.653 0.596
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Obs_Allele_Freq2(SNPSet = df_filt, ChromosomeValue = 1, threshold = .50)
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Low Bulk Observed High Parent Allele Frequency





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5
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                                         66,69
                                                1.929
7
                                         24,24
        1 10710975 0.455 0.586
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                                                 2.053
8
          10972432 0.613 0.595
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                                         32,31
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13
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                                         59,78
                                                 2.051
15
          12617905 0.504 0.521
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                                         68,66
                                                 2.051
18
        1 12618741 0.529 0.534
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                                         61,49
                                                 2.051
19
        1 12618821 0.462 0.521
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                                         67,71
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25
        1 12620681 0.534 0.575
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26
        1 12638456 0.500 0.508
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                                         64,54
                                                 2.051
28
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29
        1 12643048 0.454 0.519
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                                         39,53
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        1 17781204 0.474 0.517
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                                                 0.915
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36
        1 23496823 0.462 0.619
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40
        1 29094283 0.477 0.541
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46
        1 39405870 0.457 0.631
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53
        1 64361116 0.531 0.504
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        1 79102571 0.456 0.560
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        1 79103031 0.513 0.510
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99
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100
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                                       47,41 1.825
101
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103
        1 79104058 0.505 0.504
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111
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132
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139
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