Next-generation sequencing bulk segregant analysis with QTLseqr

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Contents

Current version: Development - 0.5.3

Standard workflow

If you use QTLseqr in published research, please cite:

> Mansfeld B.N. and Grumet R, QTLseqr: An R package for bulk segregant analysis with next-generation sequencing *bioRxiv* 208140; doi: https://doi.org/10.1101/208140

Quick Start

Here are the basic steps required to run and plot QTLseq and G' analysis

```
#load the package
library("QTLseqr")

#Set sample and file names
HighBulk <- "SRR834931"
LowBulk <- "SRR834927"
file <- "SNPs_from_GATK.table"

#Choose which chromosomes will be included in the analysis (i.e. exclude smaller contigs)
Chroms <- paste0(rep("Chr", 12), 1:12)

#Import SNP data from file
df <-
    importFromGATK(
        file = file,
```

```
        highBulk = HighBulk,
        lowBulk = LowBulk,
        chromList = Chroms
     )

#Filter SNPs based on some criteria
df_filt <-
    filterSNPs(
        SNPset = df,
        refAlleleFreq = 0.20,
        minTotalDepth = 100,
        maxTotalDepth = 400,
        minSampleDepth = 40,
        minGQ = 99
    )


#Run G' analysis
df_filt <- runGprimeAnalysis(
    SNPset = df_filt,
    windowSize = 1e6,
    outlierFilter = "deltaSNP")

#Plot
plotQTLStats(SNPset = df_filt, var = "deltaSNP", plotThreshold = TRUE, q = 0.01)
plotQTLStats(SNPset = df_filt, var = "Gprime", plotThreshold = TRUE, q = 0.01)

#export summary CSV
getQTLTable(SNPset = df_filt, alpha = 0.01, export = TRUE, fileName = "my_BSA_QTL.csv")
```

Input data

QTLseqr currently only supports table format SNP data exported from the VariantsToTable function built in to GATK. We hope to support import from any VCF file soon.

Importing SNPs from GATK

Working directly with the GATK best practices guide for whole genome sequence should result in a VCF that is compatible with QTLseqr. In general the workflow suggested by GATK is per-sample variant calling followed by joint genotyping across samples. This will produce a VCF file that includes **BOTH** bulks, each with a different sample name (here SRR834927 and SRR834931), one SNP for example:

CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	SRR834927	SRR834931
Chr1	31071	.	A	G	1390.44	PASS	..*...	GT:AD:DP:GQ:PL	0/1:34,36:70:99:897,0,855	0/1:26,22:48:99:522,0,698

**info column removed for brevity*

GATK have provided a fast VCF parser, the VariantsToTable tool, that extracts the necessary fields for easy use in downstream analysis.

We highly recommend reading What is a VCF and how should I interpret it? for more information on GATK VCF Fields and Genotype Fields

Though the use of GATK's VariantsToTable function is out of the scope of this vignette, the syntax for use with QTLseqr should look something like this:

```
java -jar GenomeAnalysisTK.jar \
-T VariantsToTable \
-R ${REF} \
-V ${NAME} \
-F CHROM -F POS -F REF -F ALT \
-GF AD -GF DP -GF GQ -GF PL \
-o ${NAME}.table
```

Where `${REF}` is the reference genome file and `${NAME}` is VCF file you wish to parse.

To run QTLseqr successfully, the required VCF fields `(-F)` are CHROM (Chromosome) and POS (Position). the required Genotype fields `(-GF)` are AD (Allele Depth), DP (Depth). Recommended fields are REF (Reference allele) and ALT (Alternative allele) Recommended Genotype fields are PL (Phred-scaled likelihoods) and GQ (Genotype Quality).

Import function

Let's install and load the QTLseqr package:

```
#Install step if you have not done so yet:
#devtools::install_github("bmansfeld/QTLseqr")
library("QTLseqr")
```

The `importFromGATK` function imports SNP data from the output of the VariantsToTable function in GATK. After importing the data, the function then calculates total reference allele frequency for both bulks together, the SNP index for each bulk, and the delta SNP index.

To demonstrate the use of this function we will load the Yang et al. (2013) data file. We first need to download the package that contains the data from github.

```
#download and load data package (~50Mb)
devtools::install_github("bmansfeld/Yang2013data")
library("Yang2013data")

#Import the data
rawData <- system.file(
    "extdata",
    "Yang_et_al_2013.table",
    package = "Yang2013data",
    mustWork = TRUE)
```

If you have your own data you can simply refer to it directly:

```
rawData <- "C:/PATH/TO/MY/DIR/My_BSA_data.table"
```

We define the sample name for each of the bulks. This should correspond to the sample names in the VCF returned by GATK. We also define a vector of the chromosomes to be included in the analysis (i.e. exclude smaller contigs), In this case, Chr1, Chr2 ... Chr12.

```
HighBulk <- "SRR834931"
LowBulk <- "SRR834927"
Chroms <- paste0(rep("Chr", 12), 1:12)
```

We then use the `importFromGATK` function to import the raw data. After importing the data, the function then calculates total reference allele frequency for both bulks together, the *SNP-index* for each SNP in each

bulk and the $\Delta SNP\text{-}index$ and returns a data frame.

$$Reference\ allele\ frequency = \frac{Ref\ allele\ depth_{HighBulk} + Ref\ allele\ depth_{LowBulk}}{Total\ read\ depth\ for\ both\ bulks}$$

$$SNP\text{-}index_{per\ bulk} = \frac{Alternate\ allele\ depth}{Total\ read\ depth}$$

$$\Delta SNP\text{-}index = SNP\text{-}index_{HighBulk} - SNP\text{-}index_{LowBulk}$$

Let's import

```
#import data
df <-
    importFromGATK(
        file = rawData,
        highBulk = HighBulk,
        lowBulk = LowBulk,
        chromList = Chroms
     )
```

```
## Warning: package 'bindrcpp' was built under R version 3.3.3
```

Loaded data frame

The loaded data frame should look like this:

```
head(df)
```

```
##   CHROM   POS REF ALT AD_REF.LOW AD_ALT.LOW DP.LOW GQ.LOW     PL.LOW
## 1  Chr1 31071   A   G         34         36     70     99  897,0,855
## 2  Chr1 31478   C   T         34         52     86     99 1363,0,844
## 3  Chr1 33667   A   G         20         48     68     99 1331,0,438
## 4  Chr1 34057   C   T         38         40     78     99 1059,0,996
## 5  Chr1 35239   A   C         25         36     61     99  987,0,630
## 6  Chr1 38389   T   C         36         42     78     99 1066,0,906
##   SNPindex.LOW AD_REF.HIGH AD_ALT.HIGH DP.HIGH GQ.HIGH     PL.HIGH
## 1    0.5142857          26          22      48      99   522,0,698
## 2    0.6046512          40          34      74      99  848,0,1099
## 3    0.7058824          24          29      53      99   765,0,599
## 4    0.5128205          29          26      55      99   673,0,772
## 5    0.5901639          40          60     100      99 1632,0,1015
## 6    0.5384615          42          40      82      99  984,0,1105
##   SNPindex.HIGH   REF_FRQ     deltaSNP
## 1     0.4583333 0.5084746 -0.055952381
## 2     0.4594595 0.4625000 -0.145191703
## 3     0.5471698 0.3636364 -0.158712542
## 4     0.4727273 0.5037594 -0.040093240
## 5     0.6000000 0.4037267  0.009836066
## 6     0.4878049 0.4875000 -0.050656660
```

Let's review the column headers:

- CHROM - The chromosome this SNP is in
- POS - The position on the chromosome in nt
- REF - The reference allele at that position

- ALT - The alternate allele
- DP.HIGH - The read depth at that position in the high bulk
- AD_REF.HIGH - The allele depth of the reference allele in the high bulk
- AD_ALT.HIGH - The alternate allele depth in the the high bulk
- GQ.HIGH - The genotype quality score, (how confident we are in the genotyping)
- SNPindex.HIGH - The calculated SNP-index for the high bulk
- Same as above for the low bulk
- REF_FRQ - The reference allele frequency as defined above
- deltaSNP - The $\Delta SNP\text{-}index$ as defined above

Filtering SNPs

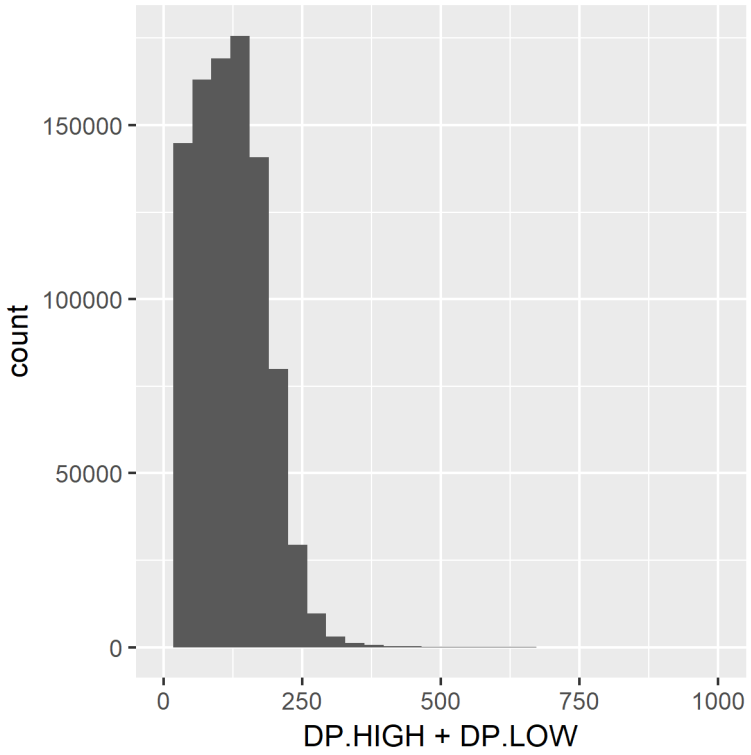
Now that we have loaded the data into R we can start cleaning it up by filtering some of the low confidence SNPs. While GATK has its own filtering tools, QTLseqr offers some options for filtering that may help reduce noise and improve results. Filtering is mainly based on read depth for each SNP, such that we can try to eliminate SNPs with low confidence, due to low coverage, and SNPs that may be in repetitive regions and thus have inflated read depth.

Read depth histograms

One way to assess filtering thresholds is by plotting histograms of the read depths. We can get an idea of where to draw our thresholds. We'll use the ggplot2 package for this purpose, but you could use base R to plot as well.

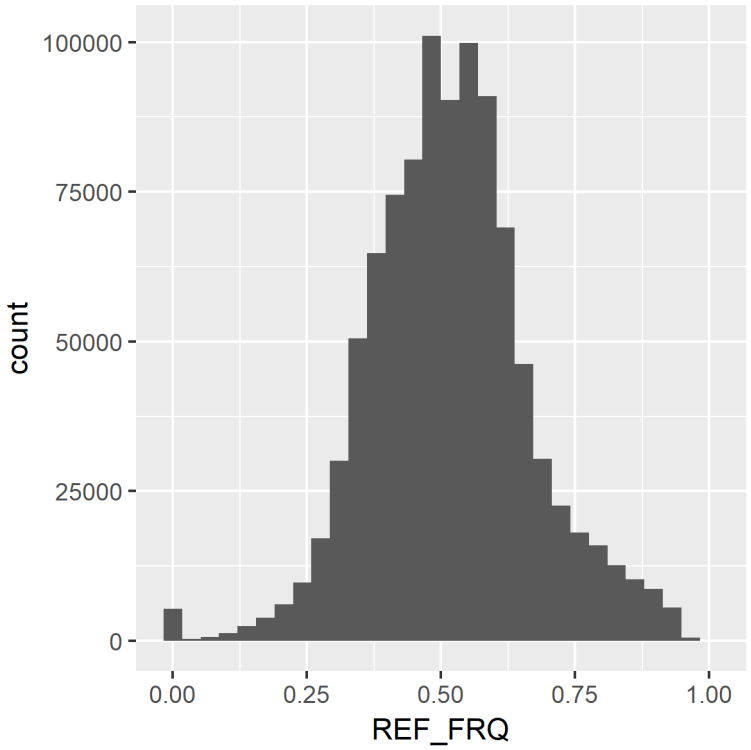
Lets look at total read depth for example:

```
library("ggplot2")
ggplot(data = df) +
    geom_histogram(aes(x = DP.HIGH + DP.LOW)) +
    xlim(0,1000)
```



. . . or look at total reference allele frequency:

```
ggplot(data = df) +
    geom_histogram(aes(x = REF_FRQ))
```



Using the filterSNPs function

Now that we have an idea about our read depth distribution we can filter out low confidence SNPS. In general we recommend filtering extremely low and high coverage SNPs, either in both bulks (`minTotalDepth/maxTotalDepth`) and/or in each bulk separately (`minSampleDepth`). We have the option to filter based on reference allele frequency (`refAlleleFreq`), this removes SNPs that for some reason are over- or under-represented in *BOTH* bulks. We can also use the GATK GQ score (Genotype Quality) to filter out low confidence SNPs. If the `verbose` parameter is set to `TRUE` (default) the function will report the numbers of SNPs filtered in each step.

```
df_filt <-
    filterSNPs(
        SNPset = df,
        refAlleleFreq = 0.20,
        minTotalDepth = 100,
        maxTotalDepth = 400,
        minSampleDepth = 40,
        minGQ = 99,
        verbose = TRUE
    )
```

```
## Filtering by reference allele frequency: 0.2 <= REF_FRQ <= 0.8
## ...Filtered 59754 SNPs
## Filtering by total sample read depth: Total DP >= 100
## ...Filtered 378814 SNPs
## Filtering by total sample read depth: Total DP <= 400
## ...Filtered 744 SNPs
## Filtering by per sample read depth: DP >= 40
## ...Filtered 4729 SNPs
## Filtering by Genotype Quality: GQ >= 99
## ...Filtered 6677 SNPs
## Original SNP number: 969487, Filtered: 450718, Remaining: 518769
```

This step is quick and we can go back and plot some histograms to see if we are happy with the results, and we can quickly re-run the filtering step if not.

Running the analysis

The analysis in QTLseqr is an implementation of both pipelines for bulk segregant analysis, G' and $\Delta SNP\text{-}index$, described by Magwene et al. (2011) and Takagi et al. (2013), respectively. We recommend reading both papers to fully understand the considerations and math behind the analysis. Here, we will briefly summarize the steps performed by the main analysis function, `runGprimeAnalysis`.

The following steps are performed:

1. First the number of SNPs within the sliding window are counted.
2. A tricube-smoothed $\Delta SNP\text{-}index$ is calculated within the set window size.
3. Genome-wide G statistics are calculated by `getG`. G is defined by the equation:

$$G = 2 * \sum n_i * ln(\frac{obs(n_i)}{exp(n_i)})$$

   Where for each SNP, n_i from i = 1 to 4 corresponds to the reference and alternate allele depths for each bulk, as described in the following table:

Allele	High Bulk	Low Bulk
Reference	n_1	n_2
Alternate	n_3	n_4

...and $obs(n_i)$ are the observed allele depths as described in the data frame. `getG` calculates the G statistic using expected values assuming read depth is equal for all alleles in both bulks:

$$exp(n_1) = \frac{(n_1 + n_2) * (n_1 + n_3)}{(n_1 + n_2 + n_3 + n_4)}$$

$$exp(n_2) = \frac{(n_2 + n_1) * (n_2 + n_4)}{(n_1 + n_2 + n_3 + n_4)}$$

$$exp(n_3) = \frac{(n_3 + n_1) * (n_3 + n_4)}{(n_1 + n_2 + n_3 + n_4)}$$

$$exp(n_4) = \frac{(n_4 + n_2) * (n_4 + n_3)}{(n_1 + n_2 + n_3 + n_4)}$$

4. G' - A tricube-smoothed G statistic is predicted by constant local regression within each chromosome using the `tricubeStat` function. This works as a weighted average across neighboring SNPs that accounts for Linkage disequilibrium (LD) while minimizing noise attributed to SNP calling errors. G values for neighboring SNPs within the window are weighted by physical distance from the focal SNP.

5. P-values are estimated based using the non-parametric method described by Magwene et al. 2011 with the function `getPvals`. Briefly, using the natural log of G' a median absolute deviation (MAD) is calculated. The G' set is trimmed to exclude outlier regions (i.e. QTL) based on Hampel's rule. An alternate method for filtering out QTL that we propose is using absolute $\Delta SNP\text{-}index$ values greater than a set threshold (default = 0.1) to filter out potential QTL. An estimation of the mode of the trimmed set is calculated using the `mlv` function from the package modeest. Finally, the mean and variance of the set are estimated using the median and mode and p-values are estimated from a log normal distribution.

6. Negative Log10- and Benjamini-Hochberg adjusted p-values are calculated using `p.adjust`.

Let's run the function:

```
df_filt <- runGprimeAnalysis(df_filt,
    windowSize = 1e6,
    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...
```

As this is window is using a tricube-smoothing kernel the window size *can* be much larger than you might expect. We however choose a window size of 1Mb for the sliding window analysis, for a discussion about window size we recommend reading Magwene et al. (2011). In general larger windows will produced smoother data. The functions making these calculations are rather fast, so we recommend testing several window sizes for your data, and deciding on the optimal size.

Some additional columns are added to the filtered data frame:

```
head(df_filt)
```

```
##   CHROM   POS REF ALT AD_REF.LOW AD_ALT.LOW DP.LOW GQ.LOW     PL.LOW
## 1  Chr1 31071   A   G         34         36     70     99  897,0,855
## 2  Chr1 31478   C   T         34         52     86     99 1363,0,844
## 3  Chr1 33667   A   G         20         48     68     99 1331,0,438
## 4  Chr1 34057   C   T         38         40     78     99 1059,0,996
## 5  Chr1 35239   A   C         25         36     61     99  987,0,630
## 6  Chr1 38389   T   C         36         42     78     99 1066,0,906
##   SNPindex.LOW AD_REF.HIGH AD_ALT.HIGH DP.HIGH GQ.HIGH     PL.HIGH
## 1    0.5142857          26          22      48      99   522,0,698
## 2    0.6046512          40          34      74      99  848,0,1099
## 3    0.7058824          24          29      53      99   765,0,599
## 4    0.5128205          29          26      55      99   673,0,772
## 5    0.5901639          40          60     100      99 1632,0,1015
## 6    0.5384615          42          40      82      99  984,0,1105
##   SNPindex.HIGH   REF_FRQ     deltaSNP nSNPs tricubeDeltaSNP          G
## 1     0.4583333 0.5084746 -0.055952381   881     -0.07365553 0.35697092
## 2     0.4594595 0.4625000 -0.145191703   881     -0.07365807 3.38161778
## 3     0.5471698 0.3636364 -0.158712542   883     -0.07367173 3.23692853
## 4     0.4727273 0.5037594 -0.040093240   883     -0.07367416 0.20748641
## 5     0.6000000 0.4037267  0.009836066   883     -0.07368154 0.01521766
## 6     0.4878049 0.4875000 -0.050656660   883     -0.07370120 0.41076961
##     Gprime    pvalue negLog10Pval    qvalue
## 1 1.919586 0.4670188    0.3306657 0.6683592
## 2 1.919650 0.4669994    0.3306837 0.6683425
## 3 1.919994 0.4668952    0.3307806 0.6682994
## 4 1.920056 0.4668766    0.3307979 0.6682904
## 5 1.920242 0.4668204    0.3308502 0.6682467
## 6 1.920737 0.4666705    0.3309896 0.6681446
```

- nSNPs - the number of SNPs bracketing the focal SNP within the set sliding window
- tricubeDeltaSNP - the tricube-smoothed $\Delta SNP\text{-}index$
- G - the G value for the SNP
- Gprime - the tricube-smoothed G value
- pvalue - the p-value calculated by non-parametric estimation
- negLog10Pval - the $-log_{10}(p\text{-}value)$
- qvalue - Benjamini-Hochberg adjusted p-values

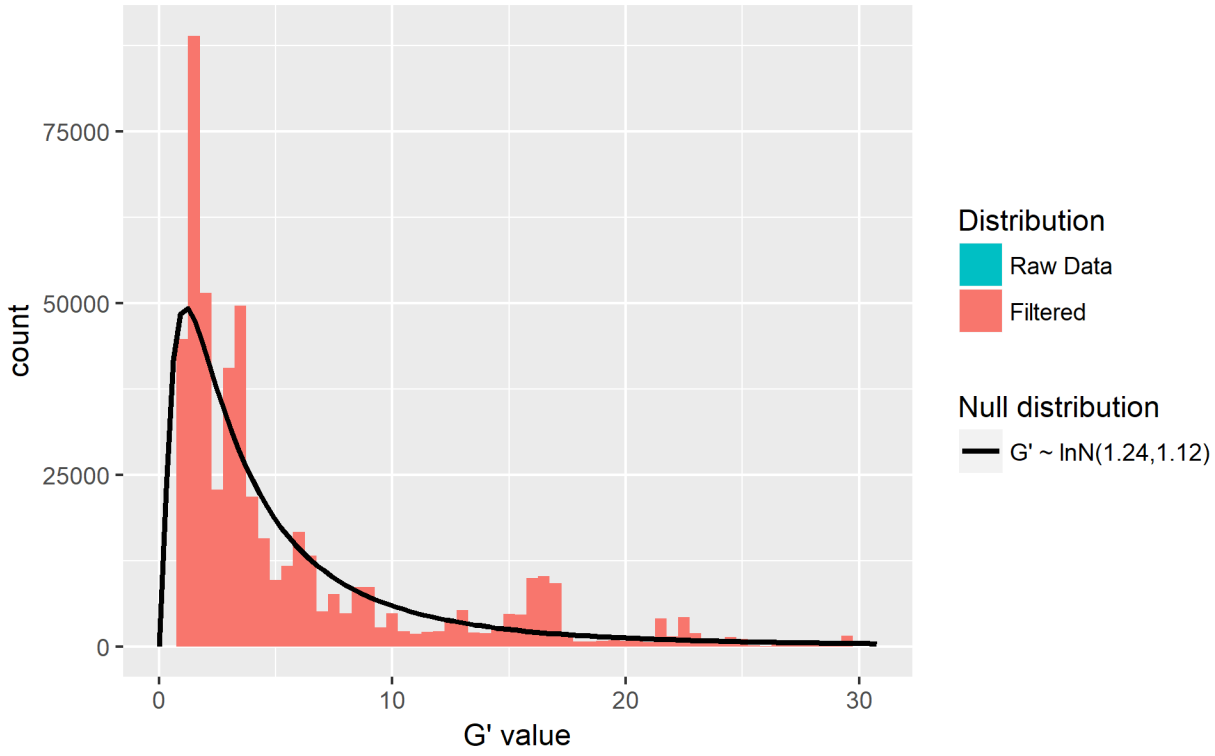
Plotting the data

QTLseqr offers two main plotting functions to check the validity of the G' analysis and to plot genome-wide or chromosome specific QTL analysis plots.

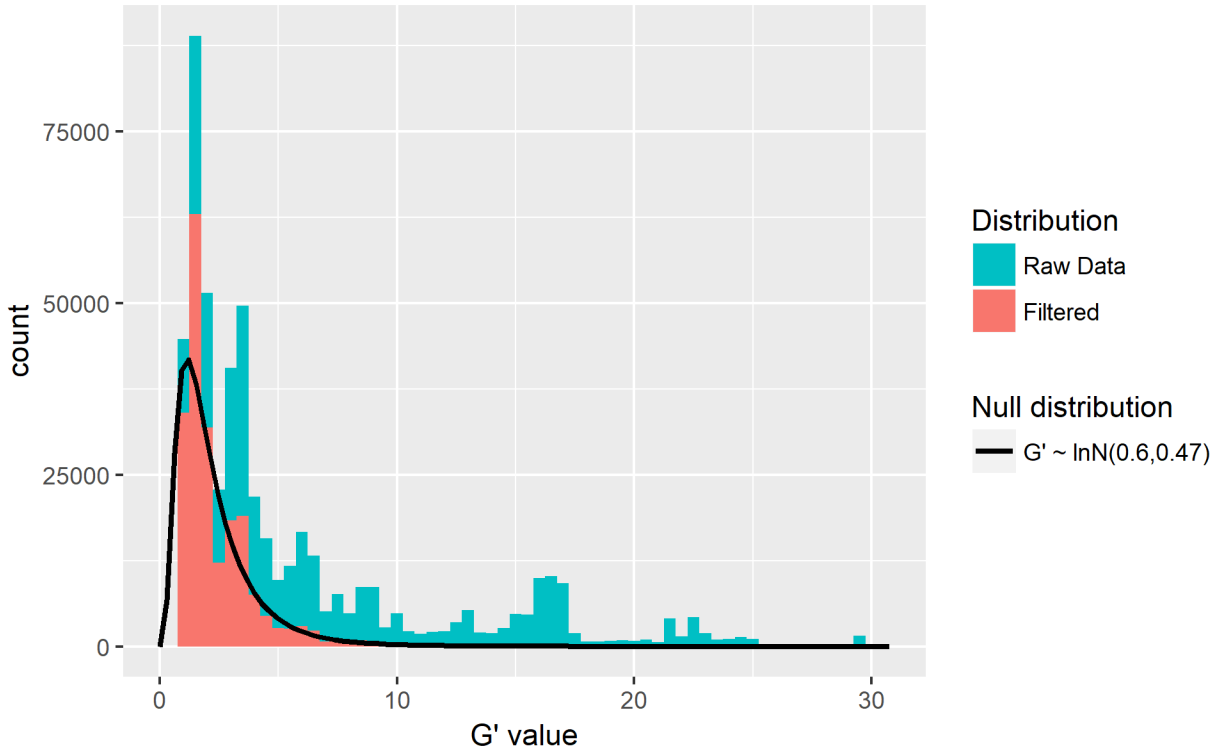
G' distribution plots

Due to the fact that p-values are estimated from the null distribution of G', an important check is to see if G' values are close to log normally distributed. For this purpose we use the `plotGprimeDist` function, which plots the G' histograms of both raw and filtered G' sets (see P-value calculation above) alongside the log-normal null distribution (which is reported in the legend). We can also use this to test which filtering method (Hampel or DeltaSNP) estimates a more accurate null distribution. If you use the `"deltaSNP"` method plotting G' distributions with different filter thresholds might also help reveal a better G' null distribution.

```
plotGprimeDist(SNPset = df_filt, outlierFilter = "Hampel")
```




```
plotGprimeDist(SNPset =df_filt, outlierFilter = "deltaSNP", filterThreshold = 0.1)
```

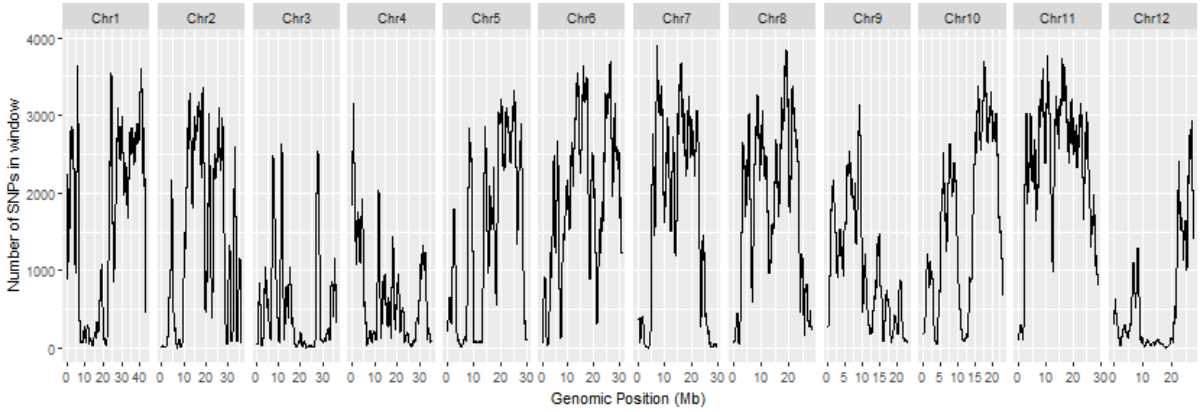


QTL analysis plots

Now that we are happy with our filtered data and it seems that the G' distribution is close to log-normal, we can finally plot some genome-wide figures and try to identify QTL.

Let's start by plotting the SNP/window distribution:

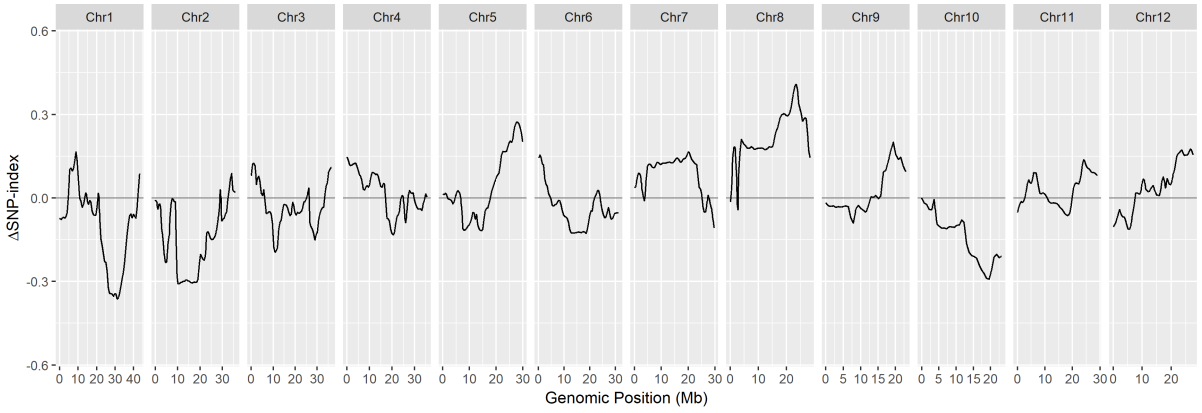
```
p1 <- plotQTLStats(SNPset = df_filt, var = "nSNPs")
p1
```



This is informative as we can assess if there are regions with extremely low SNP density.

More importantly lets identify some QTL by plotting the smoothed $\Delta SNP\text{-}index$ and G' values.

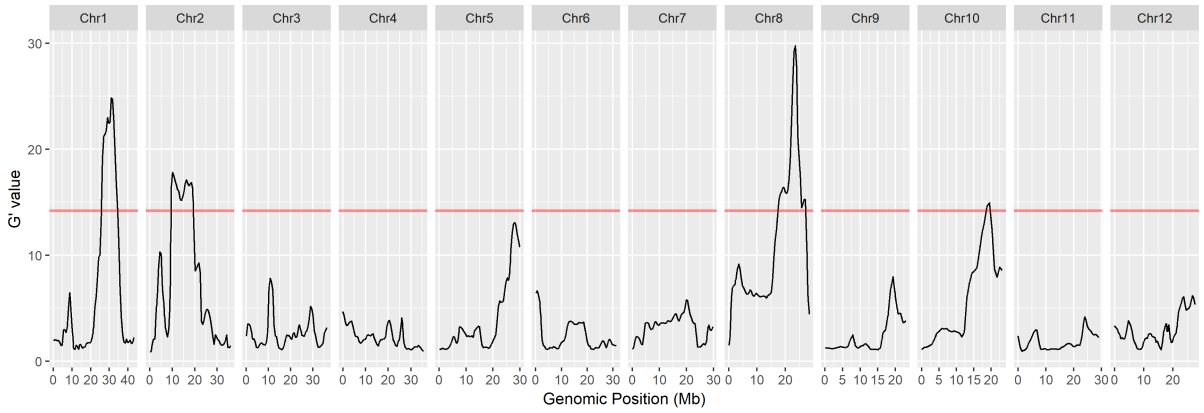
```
p2 <- plotQTLStats(SNPset = df_filt, var = "deltaSNP")
p2
```



We can see that there are some regions that have $|\Delta SNP\text{-}index| > 0.3$ these may be QTL. The directionality of the $\Delta SNP\text{-}index$ is also important. If the allele contributing to the trait is from the reference parent the $\Delta SNP\text{-}index$ should be less than 0. However, if the $\Delta SNP\text{-}index > 0$ then the contributing parent is the one with the alternate alleles.

Let's look at the G' values to see if these regions are significant and pass the FDR (q) of 0.01.

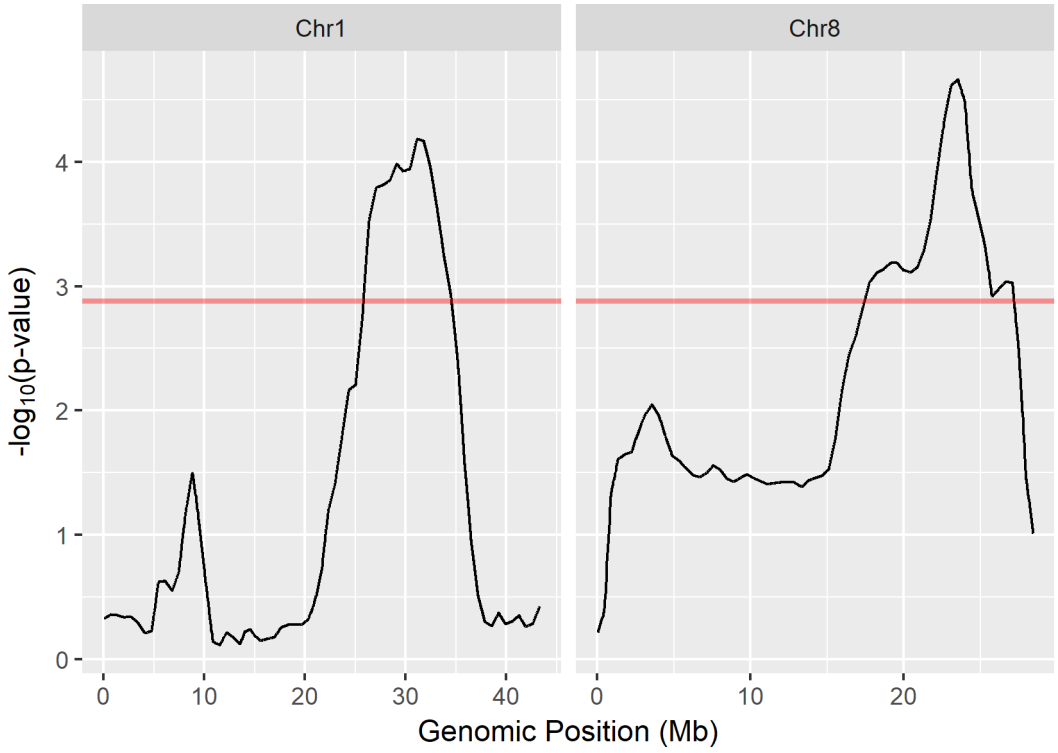
```
p3 <- plotQTLStats(SNPset = df_filt, var = "Gprime", plotThreshold = TRUE, q = 0.01)
p3
```



Great! It looks like there are QTL identified on Chromosomes 1, 2, 5, 8 and 10. Based on the $\Delta SNP\text{-}index$ and G' plots the QTL from Chr1 originates from the reference parent (Nipponbare rice, in this case) and the QTL on Chr8 was contributed by the other parent, for example.

We can also use the `plotQTLStats` function to the $-log_{10}(p\text{-}value)$. While this number is a direct derivative of G' it can be more self explanatory for some. We can use the subset parameter to plot one or a few of the chromosomes, say for a close up figure of a QTL of interest. Here we look at the $-log_{10}(p\text{-}value)$ plots of Chromosomes 1 and 8:

```
QTLplots <- plotQTLStats(
    SNPset = df_filt,
    var = "negLog10Pval",
    plotThreshold = TRUE,
    q = 0.01,
    subset = c("Chr1", "Chr8")
    )
QTLplots
```



Extracting QTL data

Now that we've plotted and identified some putative QTL we can extract the data using two functions `getSigRegions` and `getQTLTable`.

Extracting significant regions

The `getSigRegions` function will produce a list in which each element represents a QTL region. The elements are subseted from the original data frame you supplied. Any contiguous region above with an adjusted p-value above the set alpha will be returned. If there is a dip below the alpha this region will be split to two elements.

Let's examine the `head` of the first QTL:

```
QTL <- getSigRegions(SNPset = df_filt, alpha = 0.01)
head(QTL[[1]])
```

```
##       CHROM      POS REF ALT AD_REF.LOW AD_ALT.LOW DP.LOW GQ.LOW
## 24895  Chr1 25842439   T   C         21         59     80     99
## 24896  Chr1 25844940   A   G         20         59     79     99
## 24897  Chr1 25846683   A   G         16         47     63     99
## 24898  Chr1 25847043   G   A         26         61     87     99
## 24899  Chr1 25849237   G   A         12         46     58     99
## 24900  Chr1 25851646   A   T         12         41     53     99
##           PL.LOW SNPindex.LOW AD_REF.HIGH AD_ALT.HIGH DP.HIGH GQ.HIGH
## 24895 1651,0,439    0.7375000          58          47     105      99
## 24896 1675,0,399    0.7468354          74          52     126      99
## 24897 1342,0,326    0.7460317          48          30      78      99
```

```
## 24898 1679,0,563    0.7011494          90          90     180      99
## 24899 1311,0,222    0.7931034          30          20      50      99
## 24900 1150,0,227    0.7735849          35          26      61      99
##           PL.HIGH SNPindex.HIGH   REF_FRQ   deltaSNP nSNPs tricubeDeltaSNP
## 24895 1189,0,1546     0.4476190 0.4270270 -0.2898810  1068      -0.2762683
## 24896 1270,0,1984     0.4126984 0.4585366 -0.3341370  1065      -0.2764951
## 24897  768,0,1329     0.3846154 0.4539007 -0.3614164  1055      -0.2766532
## 24898 2369,0,2351     0.5000000 0.4344569 -0.2011494  1055      -0.2766858
## 24899   499,0,826     0.4000000 0.3888889 -0.3931034  1054      -0.2768848
## 24900   650,0,915     0.4262295 0.4122807 -0.3473554  1052      -0.2771032
##                G   Gprime      pvalue negLog10Pval      qvalue
## 24895 15.998496 14.52061 0.001185737     2.926012 0.009145189
## 24896 22.572856 14.54205 0.001177278     2.929121 0.009096584
## 24897 18.929521 14.55700 0.001171423     2.931286 0.009060519
## 24898  9.885982 14.56008 0.001170218     2.931733 0.009052934
## 24899 17.901883 14.57889 0.001162903     2.934457 0.009008602
## 24900 14.579060 14.59955 0.001154930     2.937444 0.008962478
```

Output QTL summary

While `getSigRegions` is useful for examining every SNP within each QTL and perhaps for some downstream analysis, the `getQTLTable` will summarize those results and can output a CSV by setting `export = TRUE` and `fileName = "MyQTLsummary.csv"`.

Here is the summary for significant regions with a FDR of 0.01:

```
results <- getQTLTable(SNPset = df_filt, alpha = 0.01, export = FALSE)
results
```

```
##   id chromosome    start      end  length nSNPs avgSNPs_Mb peakDeltaSNP
## 1  1       Chr1 25842439 34556923 8714484 20263       2325   -0.3631140
## 2  2       Chr2  9575918 19411590 9835672 24267       2467   -0.3081058
## 3  3       Chr8 17427057 27196250 9769193 20529       2101    0.4082090
## 4  4      Chr10 18619373 19792840 1173467  3590       3059   -0.2922478
##   maxGprime meanGprime  sdGprime      AUCaT     meanPval    meanQval
## 1  24.83453   21.29574 2.6768852 60704185.7 0.0002362947 0.005050808
## 2  17.82461   16.44684 0.6348595 22256829.6 0.0006525797 0.007346565
## 3  29.75614   18.89438 4.6368929 46533829.4 0.0005309317 0.006606070
## 4  14.95028   14.68470 0.2098474   577793.3 0.0011258224 0.008877309
```

The columns are:

- id - the QTL identification number
- chromosome - The chromosome on which the region was identified
- start - the start position on that chromosome, i.e. the position of the first SNP that passes the FDR threshold
- end - the end position
- length - the length in base pairs from start to end of the region
- nSNPs - the number of SNPs in the region
- avgSNPs_Mb - the average number of SNPs/Mb within that region
- peakDeltaSNP - the $\Delta SNP\text{-}index$ value at the peak summit
- maxGprime - the max G' score in the region
- meanGprime - the average G' score of that region
- sdGprime - the standard deviation of G' within the region
- AUCaT - the **A**rea **U**nder the **C**urve but **a**bove the **T**hreshold line, an indicator of how significant or wide the peak is

- meanPval - the average p-value in the region
- meanQval - the average adjusted p-value in the region

Summary

We've reviewed how to load SNP data from GATK and filter the data to contain high confidence SNPs. We then performed $\Delta SNP\text{-}index$ and G' analysis and calculate p-values and q-values based on the tricube-smoothed G' values. The QTL regions that pass our defined threshold can be stored as a list for further analysis or summarized as a table for publication.