GWAS_vis_vignette

Arcadio

12/13/2018

Loading up packages and functions

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB
```

```
## The following objects are masked from 'package:rJava':
##
##     anyDuplicated, duplicated, sort, unique
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind,
##     colMeans, colnames, colSums, dirname, do.call, duplicated,
##     eval, evalq, Filter, Find, get, grep, grepl, intersect,
##     is.unsorted, lapply, lengths, Map, mapply, match, mget, order,
##     paste, pmax, pmax.int, pmin, pmin.int, Position, rank, rbind,
##     Reduce, rowMeans, rownames, rowSums, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which, which.max,
##     which.min
```

```
## Loading required package: S4Vectors
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following object is masked from 'package:base':
##
##     expand.grid
```

```
## Loading required package: IRanges
```

```
## Loading required package: GenomeInfoDb
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
```

```
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.

## Loading required package: DelayedArray

## Loading required package: matrixStats

##
## Attaching package: 'matrixStats'

## The following objects are masked from 'package:Biobase':
##
##     anyMissing, rowMedians

## Loading required package: BiocParallel

##
## Attaching package: 'DelayedArray'

## The following objects are masked from 'package:matrixStats':
##
##     colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges

## The following objects are masked from 'package:base':
##
##     aperm, apply
```

Set directory, loading up start functions and rTassel

```
is_experimental <- TRUE

#set workdir for rtassel
setwd("~/myBins/bucklerlabBitbucket/rtassel/")

path_exp_tassel <- paste0(getwd(),"/inst/java/sTASSEL.jar")
path_exp_tassel_libs <- paste0(getwd(),"/inst/java/lib")

## jinit
rJava::.jinit(parameters="-Xmx6g")
.jcall(.jnew("java/lang/Runtime"), "J", "totalMemory")
```

```
## [1] 257425408
```

```
.jcall(.jnew("java/lang/Runtime"), "J", "maxMemory")
```

```
## [1] 5726797824
```

```
## Add class paths
if(is_experimental == TRUE) {
  tasselPath <- path_exp_tassel
  tasselLibs <- path_exp_tassel_libs
}

rJava::.jaddClassPath(tasselPath)
rJava::.jaddClassPath(tasselLibs)
print(.jclassPath())
```

```
## [1] "/Users/jav246/myBins/R-packages/rJava/java"
## [2] "/Users/jav246/myBins/bucklerlabBitbucket/rtassel/inst/java/sTASSEL.jar"
## [3] "/Users/jav246/myBins/bucklerlabBitbucket/rtassel/inst/java/lib"
```

```
## Source files
source("R/AllGenerics.R")
source("R/AllClasses.R")
source("R/TasselPluginWrappers.R")
source("R/PullFunctions.R")
source("R/gwasPolyObjectCreator.R")
```

Load up genotypes implementing Tassel code through rJava

```
geno_fileName <- "/Users/jav246/myBins/bucklerlabBitbucket/rtassel/data/mdp_genotype.hmp.txt"

## Make genotype table from tasses sample data
tasGenoTable <- readGenotypeTable(geno_fileName)

## Make summarized experiment from genotypetable
tas_se <- summarizeExperimentFromGenotypeTable(tasGenoTable)

tas_se
```

```
## class: RangedSummarizedExperiment
## dim: 3093 281
## metadata(0):
## assays(1): ''
## rownames: NULL
## rowData names(3): tasselIndex refAllele altAllele
## colnames(281): 33-16 38-11 ... WF9 YU796NS
## colData names(3): Sample TasselIndex
##   matrix.unlist.fourNewCols...nrow...length.fourNewCols...byrow...T.
```

```
genoDF <- GWASpolyGenoFromSummarizedExperiment(tas_se)

dim(genoDF)
```

```
## [1] 3093  284
```

```
genoDF[1:4, 1:8]
```

```
##   markerName chr     pos 33-16 38-11 4226 4722 A188
## 1    dummy-1   1  157104     0     0    0    0    0
## 2    dummy-2   1 1947984     0     2    0    2    0
## 3    dummy-3   1 2914066     0     0    0    0    0
## 4    dummy-4   1 2914171     0     0    0    0    0
```

```
markers_rrblup_mat <- apply(genoDF[,-(1:3)],1,convert.snp)

dim(markers_rrblup_mat)
```

```
## [1]  281 3093
```

```
markers_rrblup <- data.frame(genoDF[,c(1:3)], t(markers_rrblup_mat))

colnames(markers_rrblup) <- colnames(genoDF)

dim(markers_rrblup)
```

```
## [1] 3093  284
```

```
markers_rrblup[1:4, 1:8]
```

```
##   markerName chr     pos 33-16 38-11 4226 4722 A188
## 1    dummy-1   1  157104    -1    -1   -1   -1   -1
## 2    dummy-2   1 1947984    -1     1   -1    1   -1
## 3    dummy-3   1 2914066    -1    -1   -1   -1   -1
## 4    dummy-4   1 2914171    -1    -1   -1   -1   -1
```

Load phenotype data

```
###straight load as dataframe, skpping first two rows on tassel specific phenotype table format
pheno_fileName <- "/Users/jav246/myBins/bucklerlabBitbucket/rtassel/data/mdp_phenotype.txt"
phenos <- read.table(file = pheno_fileName, skip = 2, header = T, sep = "\t", na.strings = "NaN")
summary(phenos)
```

```
##       Taxa     location     EarHT            dpoll           EarDia
##  33-16  :  2   A:283    Min.   :  6.40   Min.   :52.60   Min.   :23.72
##  38-11  :  2   B:280    1st Qu.: 48.50   1st Qu.:63.50   1st Qu.:34.35
##  4226   :  2            Median : 60.20   Median :67.50   Median :37.00
##  4722   :  2            Mean   : 61.58   Mean   :67.78   Mean   :37.06
##  A188   :  2            3rd Qu.: 72.50   3rd Qu.:71.50   3rd Qu.:40.09
##  A214N  :  2            Max.   :138.80   Max.   :85.80   Max.   :49.30
##  (Other):551            NA's   :4        NA's   :7       NA's   :37
##        Q1               Q2               Q3
##  Min.   :0.0010   Min.   :0.0010   Min.   :0.0000
##  1st Qu.:0.0020   1st Qu.:0.0050   1st Qu.:0.0020
##  Median :0.0100   Median :0.5700   Median :0.0190
##  Mean   :0.1744   Mean   :0.5011   Mean   :0.3245
##  3rd Qu.:0.1205   3rd Qu.:0.9680   3rd Qu.:0.7940
##  Max.   :0.9990   Max.   :0.9980   Max.   :0.9980
##
```

```
### select sinlge location, as GWASpoly requires single entries for taxa.
phenosOneLoc <- phenos[phenos$location == "A",]
rownames(phenosOneLoc) <- phenosOneLoc$Taxa
###remove location as it is now redundant.
###Also, GWASpoly expects all traits as initial columns, and fixed effect covariates last
phenosOneLoc <- phenosOneLoc[,-c(2)]

summary(phenosOneLoc)
```

```
##       Taxa         EarHT            dpoll           EarDia
##  33-16  :  1   Min.   :  8.00   Min.   :54.50   Min.   :23.72
##  38-11  :  1   1st Qu.: 48.12   1st Qu.:64.00   1st Qu.:34.86
##  4226   :  1   Median : 60.50   Median :67.50   Median :37.32
##  4722   :  1   Mean   : 61.75   Mean   :67.75   Mean   :37.20
##  A188   :  1   3rd Qu.: 73.00   3rd Qu.:71.50   3rd Qu.:40.02
##  A214N  :  1   Max.   :136.00   Max.   :85.00   Max.   :46.35
##  (Other):277   NA's   :1        NA's   :3       NA's   :33
##        Q1               Q2               Q3
##  Min.   :0.0010   Min.   :0.001   Min.   :0.0000
##  1st Qu.:0.0020   1st Qu.:0.005   1st Qu.:0.0020
##  Median :0.0090   Median :0.579   Median :0.0230
##  Mean   :0.1728   Mean   :0.502   Mean   :0.3253
##  3rd Qu.:0.1160   3rd Qu.:0.968   3rd Qu.:0.7940
##  Max.   :0.9990   Max.   :0.998   Max.   :0.9980
```

##

Run GWAS

```
## create GWASpoly object with coopted read.GWASpoly function
#uses tassel created summarizedExperiment for genotypes
data_gwasPoly <- se_createGWASpolyObject(ploidy = 2, phenoDF = phenosOneLoc,
                                         SummarizedExperimentObject =  tas_se,
                                         format = "numeric", n.traits = 3)
```

```
## Number of polymorphic markers: 3093
## Missing marker data imputed with population mode
## N = 264 individuals with phenotypic and genotypic information
## Detected following fixed effects:
## Q1
## Q2
## Q3
## Detected following traits:
## EarHT
## dpoll
## EarDia
```

```
## add kinship information to object
data_gwasPoly <- set.K(data_gwasPoly)

## set parameters for mixed model
params <- set.params(fixed=unlist(strsplit("Q1,Q2,Q3", ",")),
                     fixed.type=rep("numeric",3))

## run gwas with GWASpoly
data_gwasPoly_res <- GWASpoly(data = data_gwasPoly, models = "additive",
                              params = params)
```

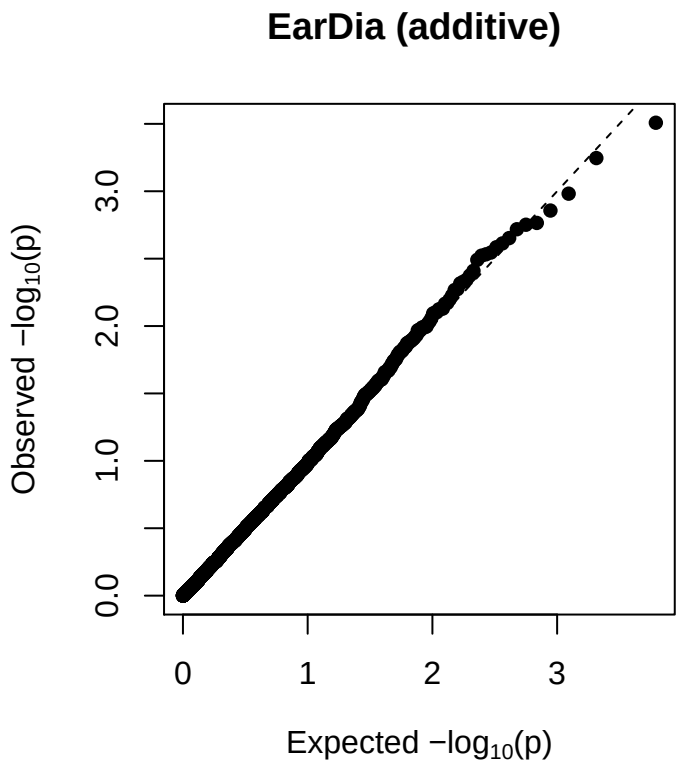
```
## Analyzing trait: EarHT
## P3D approach: Estimating variance components...Completed
## Testing markers for model: additive
## Analyzing trait: dpoll
## P3D approach: Estimating variance components...Completed
## Testing markers for model: additive
## Analyzing trait: EarDia
## P3D approach: Estimating variance components...Completed
## Testing markers for model: additive
```

```
#run gwas with rrBLUP
k_rrblup <- A.mat(markers_rrblup_mat)

phenosOneLoc_rrblup <- phenosOneLoc[phenosOneLoc$Taxa %in% colnames(markers_rrblup), ]

gwas_rrblup <- GWAS(pheno = phenosOneLoc_rrblup, geno = markers_rrblup, K = k_rrblup,
                    fixed = unlist(strsplit("Q1,Q2,Q3", ",")), P3D = T, n.core=6, plot = F)
```

```
## [1] "GWAS for trait: EarHT"
## [1] "Variance components estimated. Testing markers."
## [1] "GWAS for trait: dpoll"
## [1] "Variance components estimated. Testing markers."
## [1] "GWAS for trait: EarDia"
## [1] "Variance components estimated. Testing markers."
```

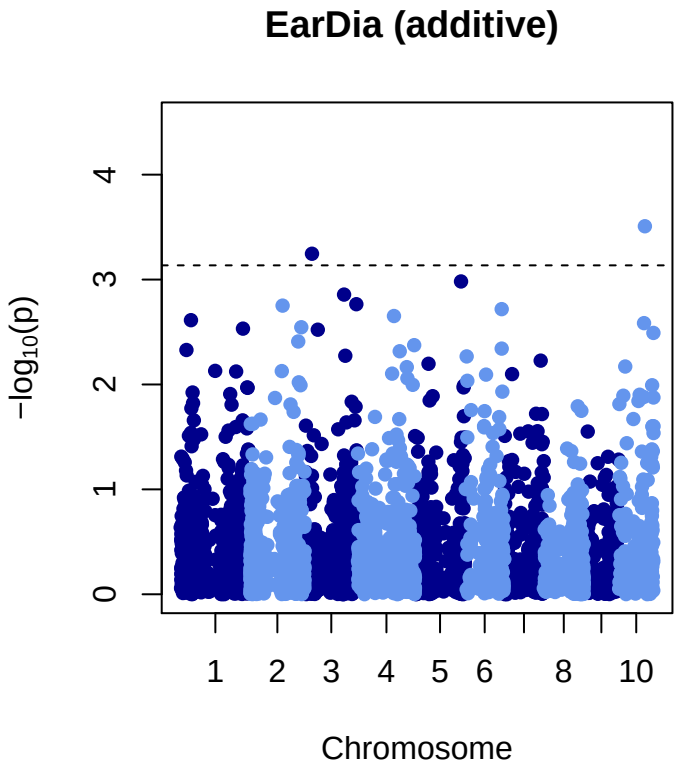
Create GWASpoly plots and set thresholds to get QTLs

```
qq.plot(data_gwasPoly_res, trait = "EarDia", model = "additive")
```




```
## NULL
```

```
#can set Bonferroni and own pvalue
data_gwasPoly_res <- set.threshold(data_gwasPoly_res, method = "FDR", level=0.05)

#can set any of the 3 traits
manhattan.plot(data_gwasPoly_res, trait = "EarDia", model = "additive")
```




```
## NULL
```

```
get.QTL(data = data_gwasPoly_res)
```

```
##       Trait    Model Threshold     Marker Chrom  Position Ref Alt Score
## 987  EarDia additive      3.14  dummy-987     3  31695534   0   1  3.25
## 2987 EarDia additive      3.14 dummy-2987    10 111608788   0   1  3.51
##      Effect
## 987   -1.08
## 2987  -1.15
```

```
#issue with gwasPoly output.
#Effect sizes of QTL don't match the self-contained effect sizes
data_gwasPoly_res@scores$EarHT[rownames(data_gwasPoly_res@scores$EarDia) %in%
                                 get.QTL(data = data_gwasPoly_res)$Marker,1]
```

```
## [1] 0.8661518 0.5684213
```

```
#when checking with my code for unwrapping the gwas poly output
#you can find that there are effectively two QTL
#with the effect sizes that the self reported gwaspoly shows
#but not in the position that they were supposed to be
traitGWASresults <- gwasPolyToDF(data_gwasPoly_res)
```

```
## getting results for: EarHT
```

```
## getting results for: dpoll
```

```
## getting results for: EarDia
```

```
traitGWASresults[traitGWASresults$markerLogPVal > traitGWASresults$sigTreshold,]
```

```
##          Marker markerpVal markerLogPVal markerEffect  trait sigTreshold
## 4036 dummy-2209 0.02995077      3.508200    -1.145989 EarDia     3.13549
```

```
## 6942 dummy-3080 0.03892190      3.246198    -1.083329 EarDia     3.13549
##      Chrom  Position Ref Alt
## 4036     7  14349767   0   1
## 6942    10 144548839   0   1
```

Unwrap gwaspoly results class object

```
traitGWASresults <- gwasPolyToDF(data_gwasPoly_res)
```

```
## getting results for: EarHT
```

```
## getting results for: dpoll
```

```
## getting results for: EarDia
```

```
traitGWASresults[traitGWASresults$markerLogPVal> traitGWASresults$sigTreshold,]
```

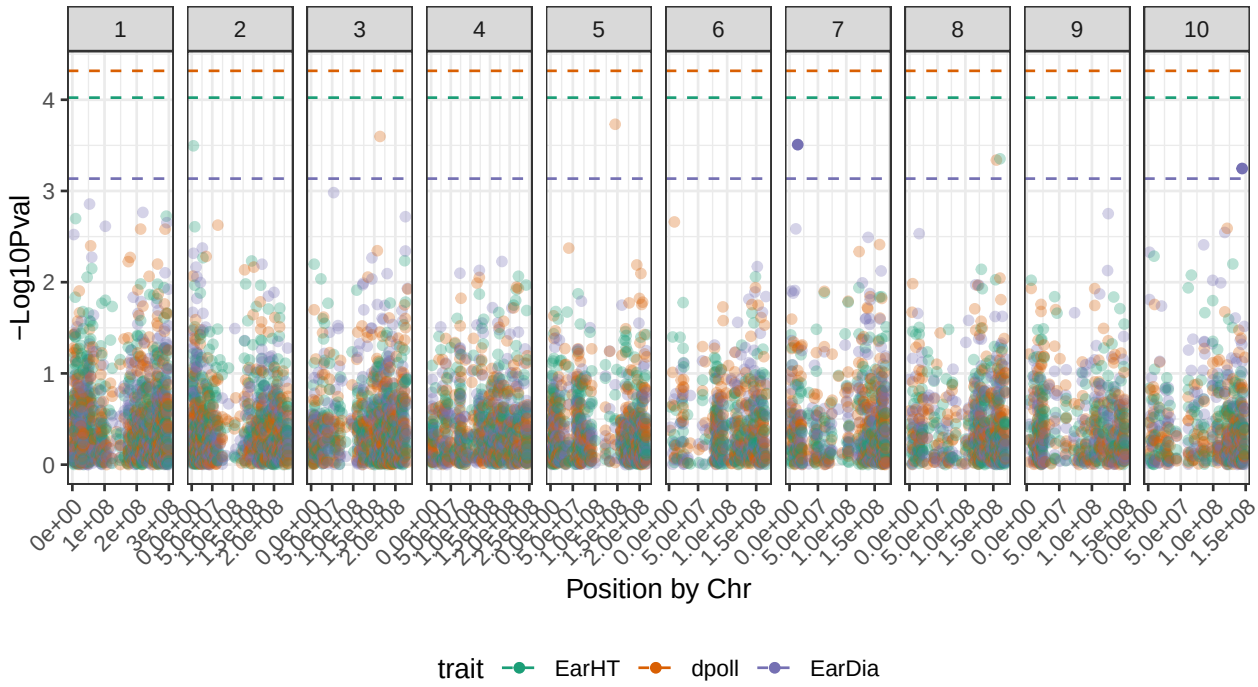
```
##          Marker markerpVal markerLogPVal markerEffect  trait sigTreshold
## 4036 dummy-2209 0.02995077      3.508200    -1.145989 EarDia     3.13549
## 6942 dummy-3080 0.03892190      3.246198    -1.083329 EarDia     3.13549
##      Chrom  Position Ref Alt
## 4036     7  14349767   0   1
## 6942    10 144548839   0   1
```

```
summary(traitGWASresults)
```

```
##         Marker       markerpVal      markerLogPVal       markerEffect
##  dummy-1   :   3   Min.   :0.02396   Min.   :0.000007   Min.   :-4.37836
##  dummy-10  :   3   1st Qu.:0.54946   1st Qu.:0.127346   1st Qu.:-0.29295
##  dummy-100 :   3   Median :0.73557   Median :0.307111   Median : 0.02879
##  dummy-1000:   3   Mean   :0.69642   Mean   :0.434249   Mean   : 0.07314
##  dummy-1001:   3   3rd Qu.:0.88043   3rd Qu.:0.598824   3rd Qu.: 0.36503
##  dummy-1002:   3   Max.   :0.99999   Max.   :3.731254   Max.   : 5.34599
##  (Other)   :9261
##     trait       sigTreshold        Chrom         Position
##  EarHT :3093   Min.   :3.135   1      :1620   Min.   :   139753
##  dpoll :3093   1st Qu.:3.135   2      :1179   1st Qu.: 43868122
##  EarDia:3093   Median :4.023   5      :1071   Median :128402775
##                Mean   :3.825   3      :1065   Mean   :119893324
##                3rd Qu.:4.317   4      : 957   3rd Qu.:175159119
##                Max.   :4.317   8      : 768   Max.   :299170077
##                                (Other):2619
##       Ref          Alt
##  Min.   :0   Min.   :1
##  1st Qu.:0   1st Qu.:1
##  Median :0   Median :1
##  Mean   :0   Mean   :1
##  3rd Qu.:0   3rd Qu.:1
##  Max.   :0   Max.   :1
##
```

Create simple manhattan like plot for all traits

```
manhattan_trait_plot(traitGWASresults = traitGWASresults, traitIDcol = "trait",
                     positionIDcol = "Position", chromIDcol = "Chrom", pValIDcol = "markerpVal")
```



Parse GFF file to get genes and create GenomicRanges object

```
maizeGFFgenesGR <- gffToGeneGR(gffFile = "~/Box/projectMaize/PHG/cimmyt_assemblies_analy/b73/Zea_mays.A
```

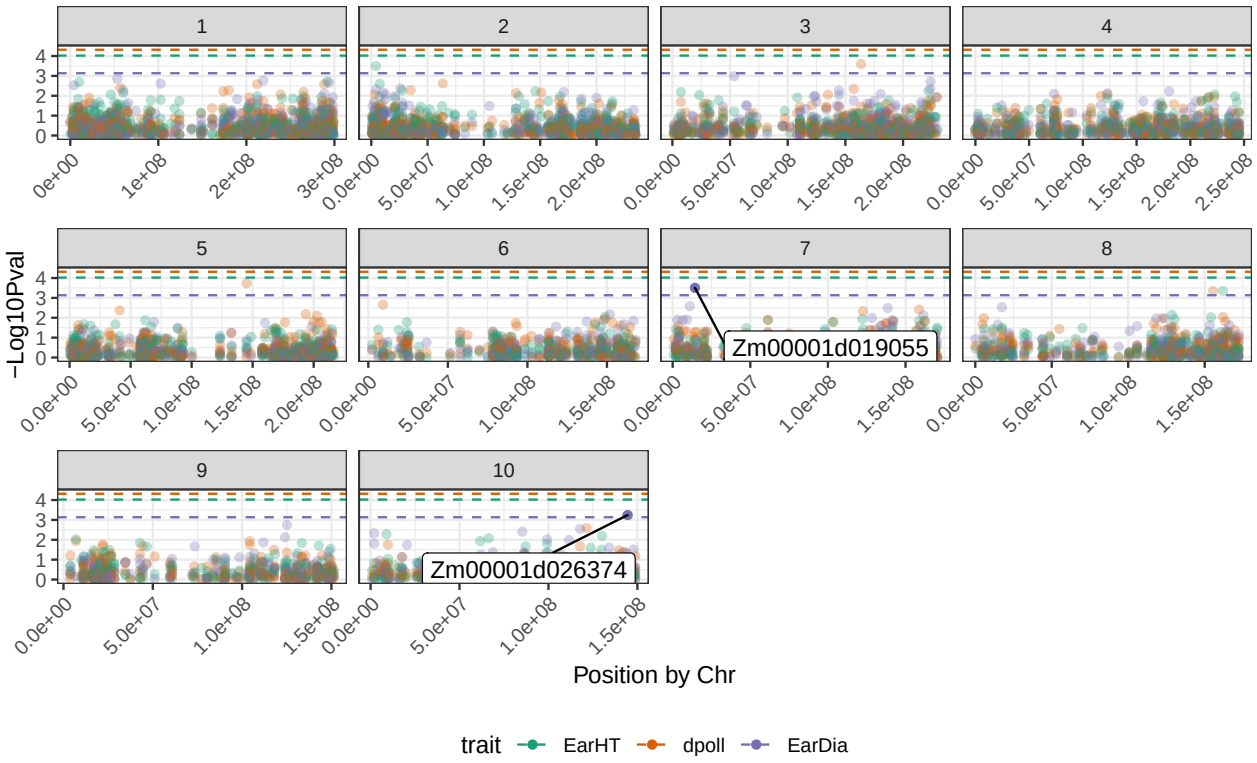
Annotating SNPs with their closest gene. Best for annotating purposes.

```
traitGWASresults_annotated <- annotate_gwasRes_byNearest(gwasResDF = traitGWASresults,
                  annotationGR = maizeGFFgenesGR, positionIDcol_gwas = "Position",
                  chromIDcol_gwas = "Chrom", outFmt = "data.frame")
```

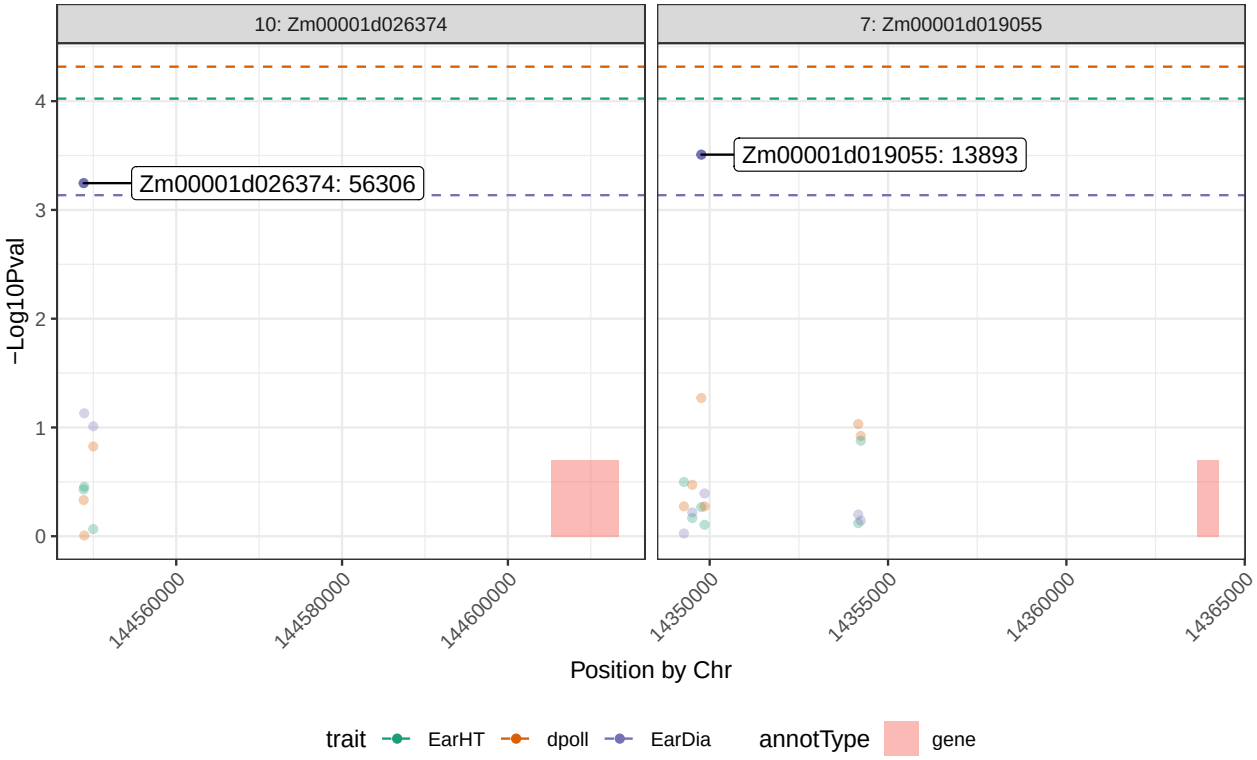
```
## Returning data.frame
```

Plot manhattan with nearest annotation on significant SNPs or by genomicRange set

```
#plotting by chromosome
manhattan_annot_plot(annotatedGWASresults = traitGWASresults_annotated,
                     labelType = "annotationName", zoomGR = "none")
```

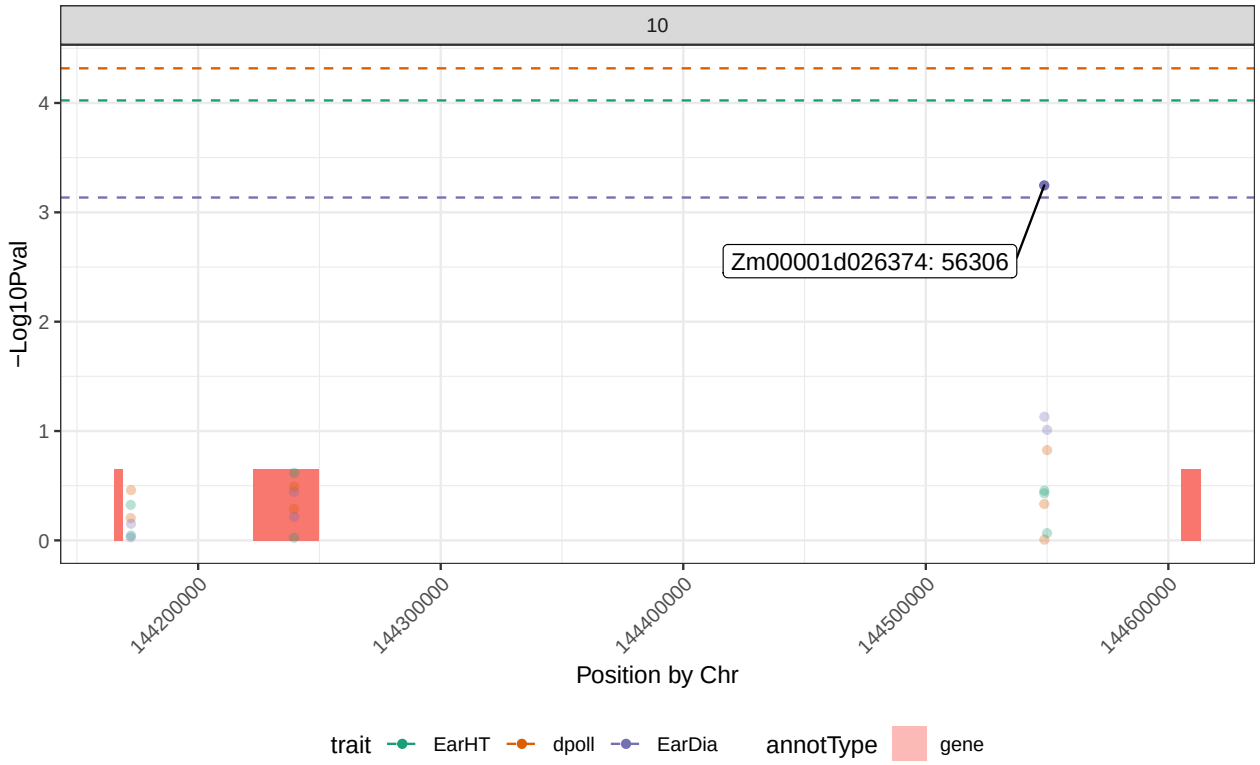



```
#plotting with zoom over each closest significant annotations
manhattan_annot_plot(annotatedGWASresults = traitGWASresults_annotated,
                     labelType = "composite", zoomGR = "auto")
```




```
#plotting with zoom over genomicRanges
#actual plotting on region still dependent on having any SNP within the defined range
```

```
myGRegions <- GRanges(seqnames = 10, ranges = IRanges(start=144605146-5e5, end=144605146+5e5))
manhattan_annot_plot(annotatedGWASresults = traitGWASresults_annotated,
                     labelType = "composite", zoomGR = myGRegions)
```



Subset GWAS plot by regions

Make GenomicRanges object out of gwas results

Subset geneRanges with gwasRanges. Finding genes with SNPs in them

Subset GWAS hits by significance for plotting with genes.