Sea lamprey RAPTURE dataset characterization

Sard et al. 2019

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Contents

Introduction and Setup

RAD capture (RAPTURE) provides a means of rapidly genotyping hundreds to thousands of standardized restriction site associated (RAD) loci. Here we provide a detailed characterization of a panel of 12,435 RAD loci developed for sea lamprey management and research. The input file for all the following analyses is appendedLoci, which is available in the research compendium (R package) described below.

Install SeaLampreyRapture package

The SeaLampreyRapture package includes all data necessary for performing the following analyses. It can be download from GitHub using the following R commands.

```
options(repos=structure(c(CRAN="http://cran.r-project.org")))
install.packages("devtools")
library(devtools)
install_github("ScribnerLab/SeaLampreyRapture")
```

Load required packages

Interactively run /analysis/installPackages.R to ensure all necessary packages are available

```
library(adegenet)
library(vcfR)
library(hierfstat)
library(tidyverse)
library(gridExtra)
library(ggthemes)
library(SeaLampreyRapture)
library(quantsmooth)
library(OutFLANK)
library(ggrepel)
library(VariantAnnotation)
```

```r
library(snpStats)
data(SeaLampreyRapture)
```

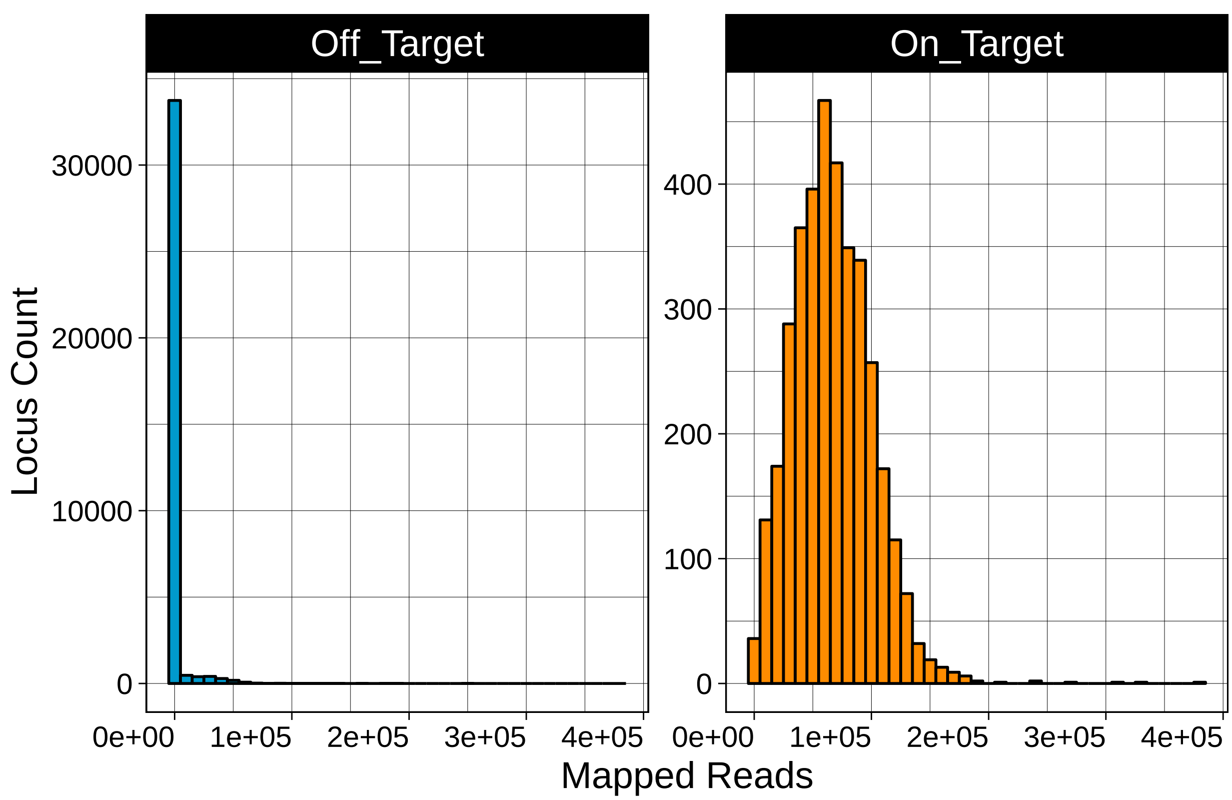
Sequencing profile

The following section characterizes the sequencing results.

On/off target reads per locus

The number of mapped read pairs for RAD loci targeted with rapture (On_Target) versus RAD loci that were not targeted (Off_Target). Overall, 80.6% of reads aligned to targeted loci even though they only made up less than 10% of all RAD loci.

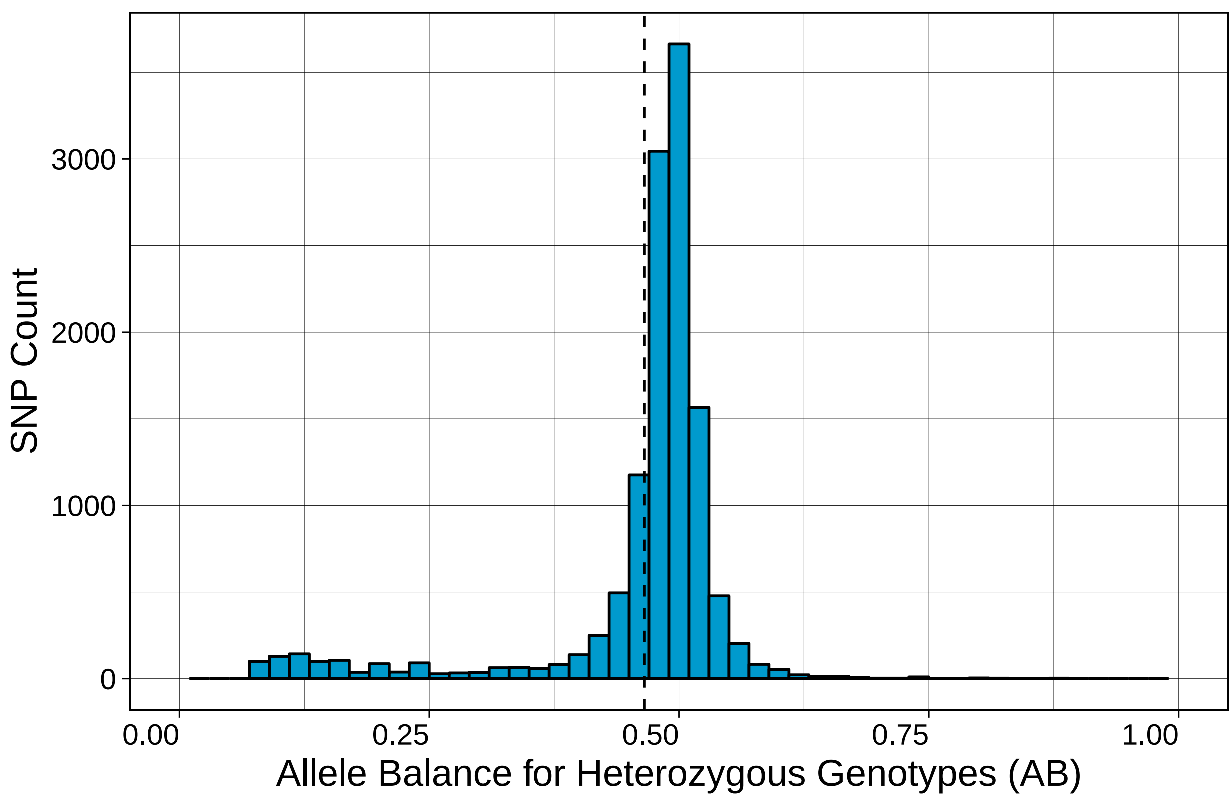
```r
p <- ggplot(onTarget_readCount, aes(x=ReadCount, fill = T)) +
  geom_histogram(binwidth = 10000, color = "black") +
  scale_fill_manual(values=c("deepskyblue3", "darkorange"))+
  facet_wrap(~T, scales = "free_y")

p + ggtitle("") +
  xlab("Mapped Reads") + ylab("Locus Count") +
  theme_linedraw() +
  theme(strip.text.x = element_text(size = 14, angle = 0)) +
  theme(legend.position="none",
        text = element_text(size=14),
        axis.text.x = element_text(angle=0, hjust=1))
```



Allele balance

Allele read balance (AB) calculated for all SNPs in manuscript Table 1. Allele balance is the proportion of reads supporting the reference allele for heterozygous genotypes at a given locus. We expect values to be near 0.5 for diploid loci that are not confounded by sequencing or mapping errors.

```
s1 <- subset(appendixLoci, allele_balance < 0.7)
s2 <- subset(s1, allele_balance > 0.3)

p <- ggplot(appendixLoci, aes(x=allele_balance)) +
  geom_histogram(fill="deepskyblue3", color = "black", binwidth = 0.02) +
  xlim(c(0,1)) +
  geom_vline(xintercept = mean(appendixLoci$allele_balance), linetype = "dashed")

p + ggtitle("") +
  xlab("Allele Balance for Heterozygous Genotypes (AB)") + ylab("SNP Count") +
  theme_linedraw()+
  theme(legend.position="none",
        text = element_text(size=14),
        axis.text.x = element_text(angle=0, hjust=1))
```

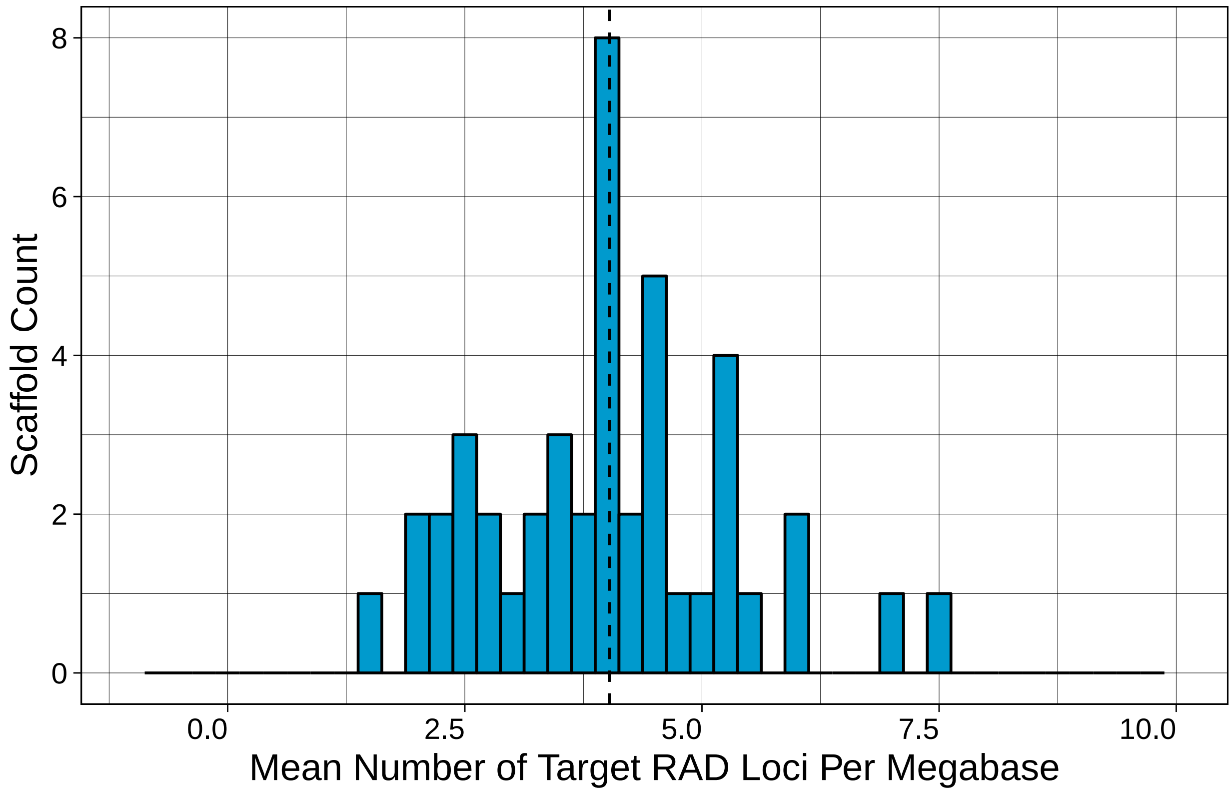


Target density

The mean density of targeted loci across these scaffolds was 4.02 RAD loci per mega-base (SD = 1.30)

```
data <- subset(targetDensity, chr_length > 10000000)
mn <- mean(data$density)
sd <- max(data$density)
```

```
p <- ggplot(data, aes(x=density)) +
  geom_histogram(fill="deepskyblue3", color = "black", binwidth = 0.25) +
  xlim(c(-1,10)) +
  geom_vline(xintercept = mn, linetype = "dashed")

p + ggtitle("") +
  xlab("Mean Number of Target RAD Loci Per Megabase") + ylab("Scaffold Count") +
  theme_linedraw()+
  theme(legend.position="none",
        text = element_text(size=14),
        axis.text.x = element_text(angle=0, hjust=1))
```

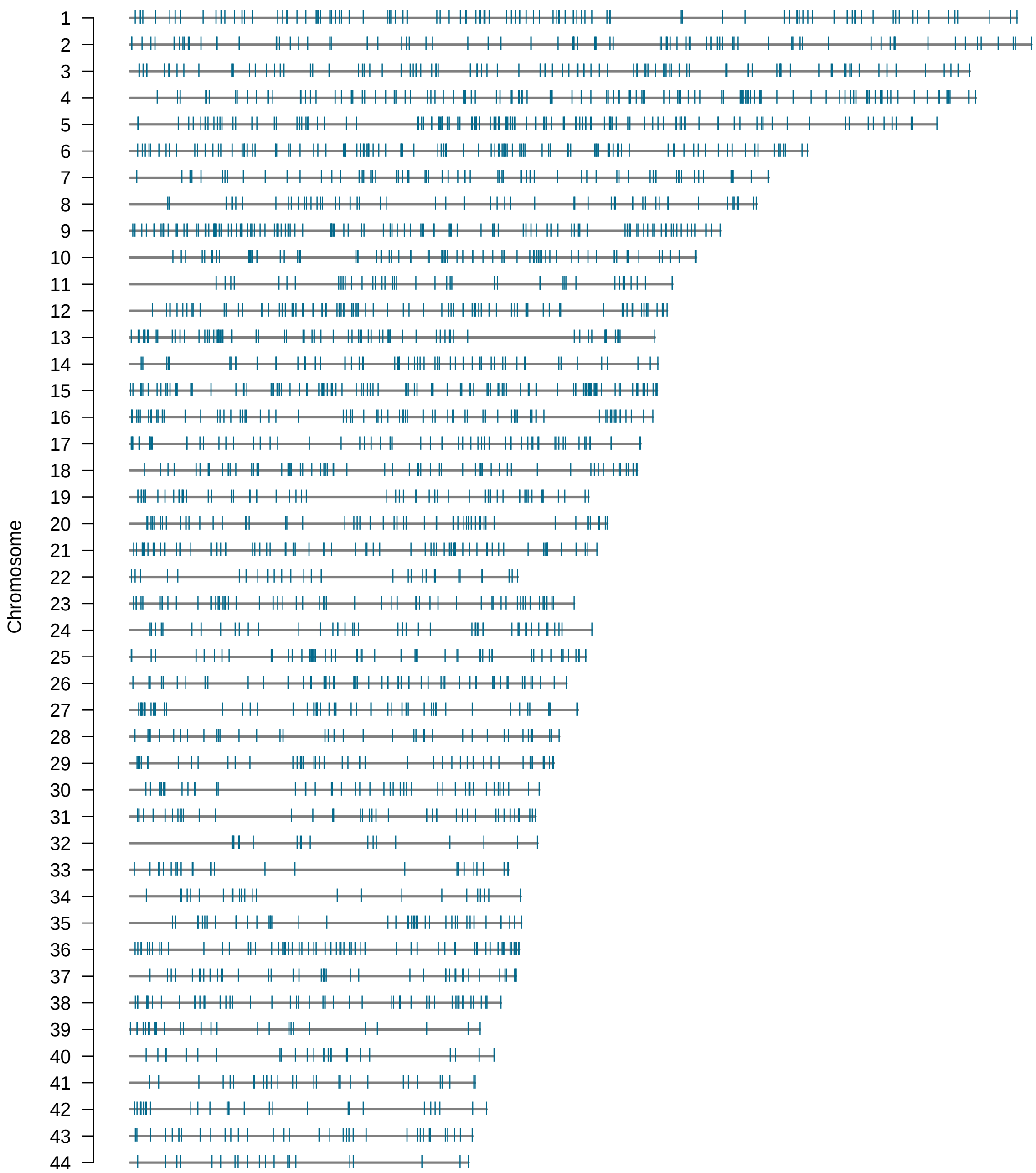


Chromosome plots

Greater than 10 mb

Distribution of target RAD loci across the 44 largest scaffolds (>10 mb) in the sea lamprey genome. Each horizontal black line represents a scaffold and each vertical blue line represents the location of a targeted locus. A total of 2844 of 3446 targeted loci map to these scaffolds (82.5%).

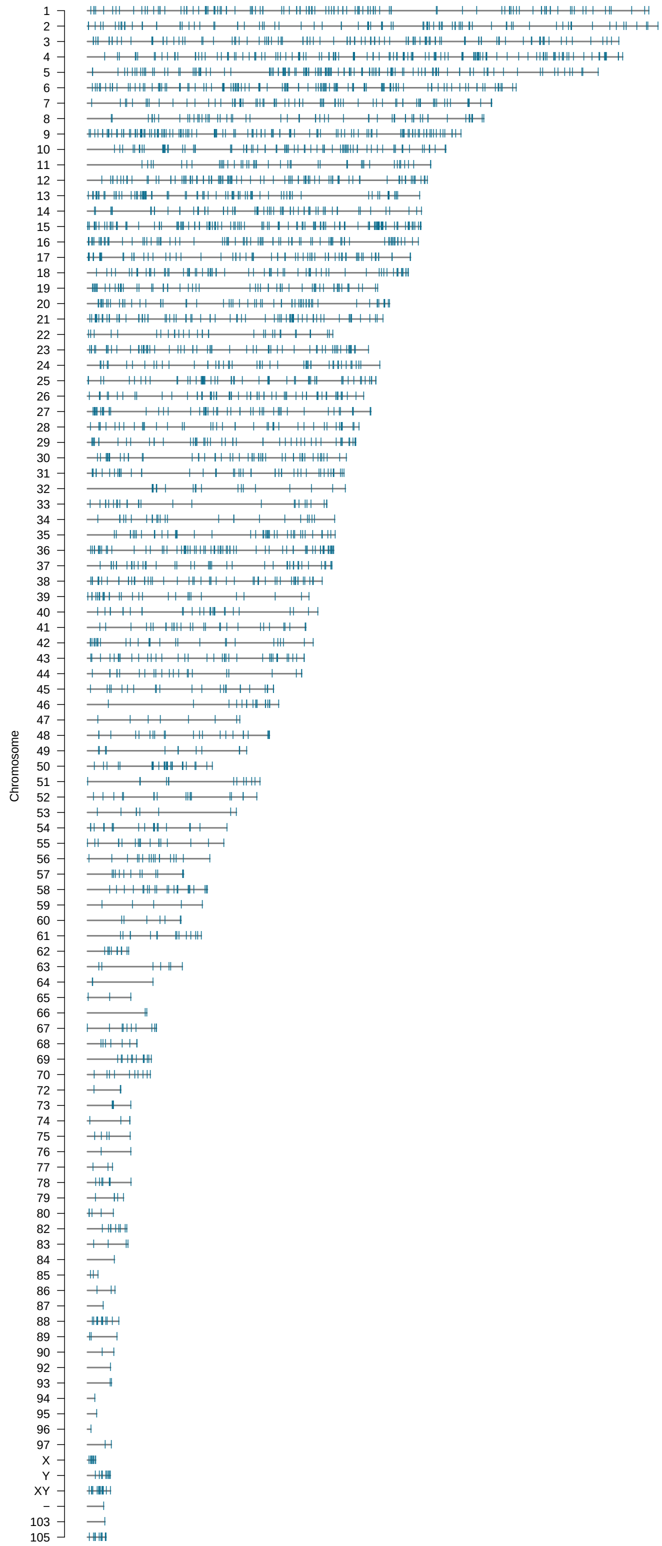
```
dat45 <- subset(targets.chrpos, targets.chrpos$CHR < 45)
chrompos <- prepareGenomePlot(dat45, cols = "grey50", paintCytobands = TRUE, bleach = 0, topspace = 1, 
points(chrompos[,2],chrompos[,1]+0.05,pch="|", cex = 0.75, col="deepskyblue4")
```



Greater than 1 mb

Distribution of target RAD loci across 100 scaffolds greater than 1 MB in length. Each horizontal black line represents a scaffold and each vertical blue line represents the location of a targeted locus. A total of 3316 of 3446 targeted loci map to these scaffolds (96.22%).

```
dat106 <- subset(targets.chrpos, targets.chrpos$CHR < 106)
chrompos <- prepareGenomePlot(dat106, cols = "grey50", paintCytobands = TRUE, bleach = 0, topspace = 1,
points(chrompos[,2],chrompos[,1]+0.05,pch="|", cex = 0.75, col="deepskyblue4")
```


Chromosome
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 72 73 74 75 76 77 78 79 80 82 83 84 85 86 87 88 89 90 92 93 94 95 96 97 X Y XY – 103 105

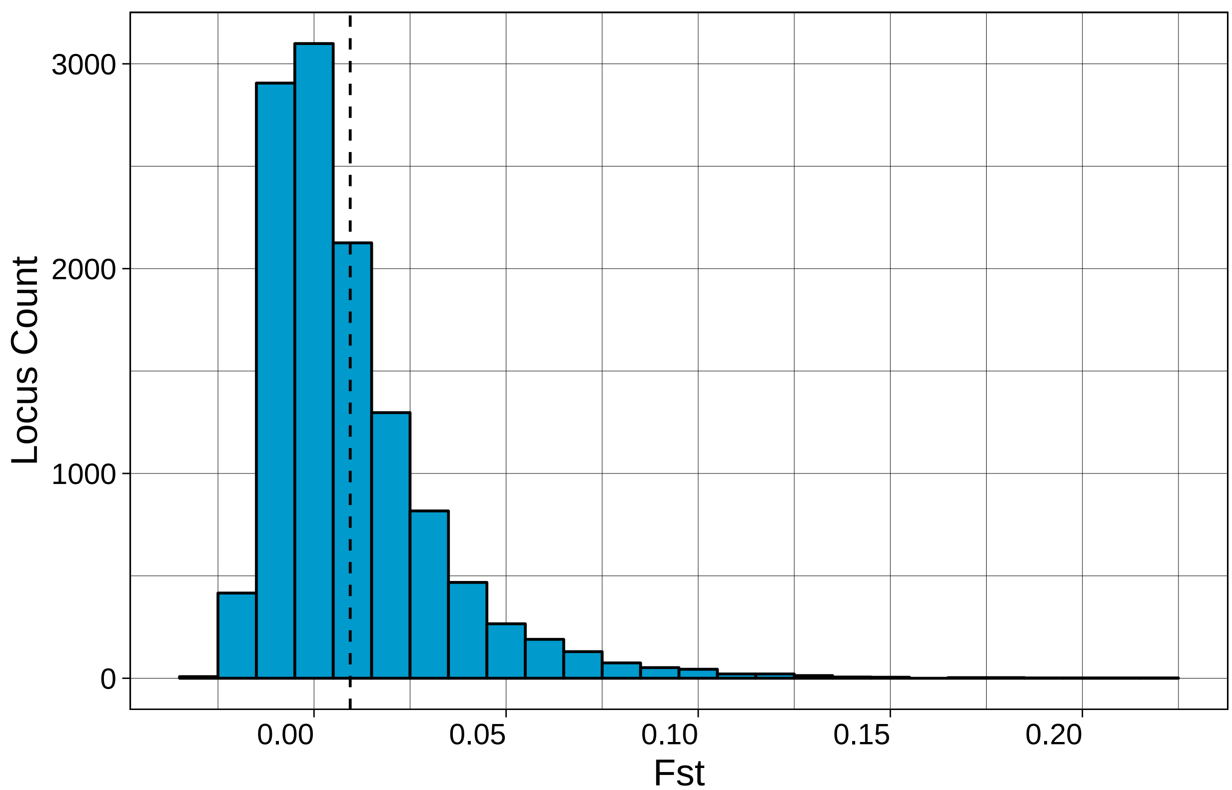
Genetic variation

The following section characterizes the genetic variation detected using the bait panel

Histogram of F_{ST} values per locus

Distribution of F_{ST} values for 11,970 SNP loci genotyped in sea lamprey at five spawning sites. The dashed vertical line indicates the mean F_{ST} value.

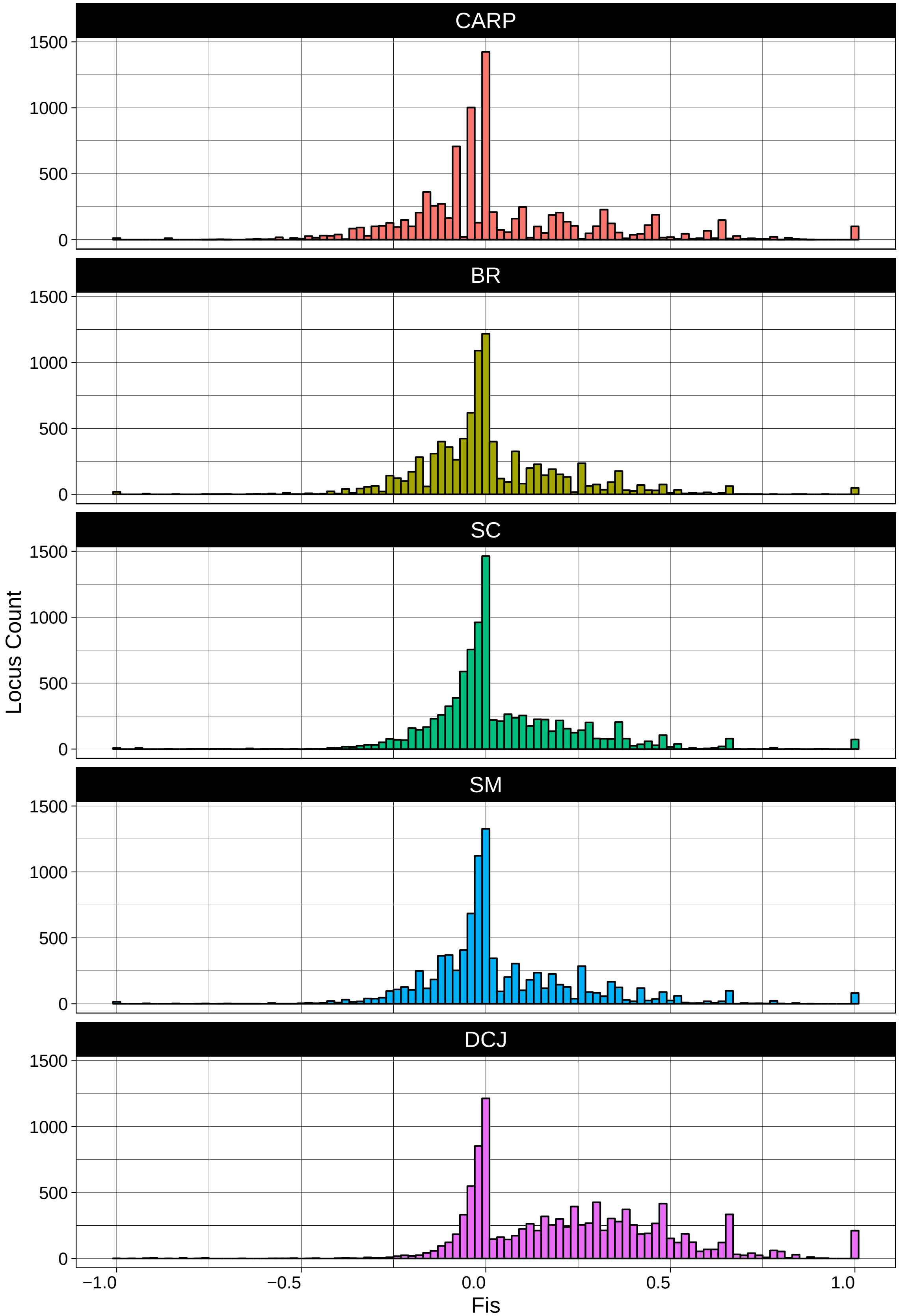
```
dat.fst <- appendixLoci %>% drop_na(fst_nei73_heirfstat)
Fst <- dat.fst$fst_nei73_heirfstat
mf <- mean(Fst)

p <- ggplot(dat.fst, aes(x=fst_nei73_heirfstat)) +
  geom_histogram(fill="deepskyblue3",color = "black", binwidth = 0.01) +
  geom_vline(xintercept = mf, linetype = "dashed")

p + ggtitle("") +
  xlab("Fst") + ylab("Locus Count") +
  theme_linedraw()+
  theme(legend.position="none",
        text = element_text(size=14),
        axis.text.x = element_text(angle=0, hjust=1))
```



F_{IS} values per locus, per population

Distributions of F_{IS} generally centered around zero for 11,970 SNP loci genotyped in sea lamprey at five spawning sites.

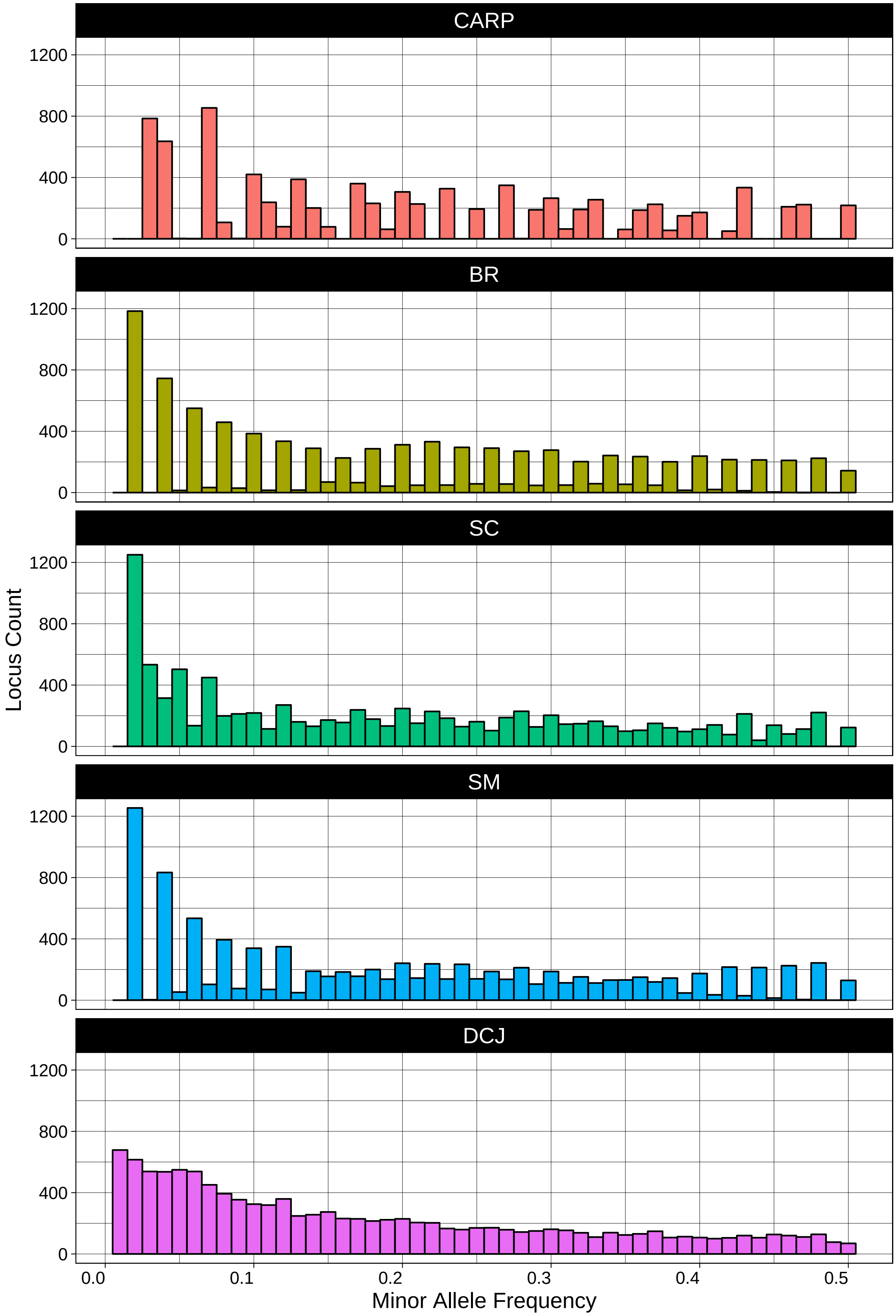
```
CARP <- as.data.frame(cbind(appendixLoci$Fis_CARP, rep(x = "CARP", nrow(appendixLoci))))
BR <- as.data.frame(cbind(appendixLoci$Fis_BR, rep(x = "BR", nrow(appendixLoci))))
SC <- as.data.frame(cbind(appendixLoci$Fis_SC, rep(x = "SC", nrow(appendixLoci))))
SM <- as.data.frame(cbind(appendixLoci$Fis_SM, rep(x = "SM", nrow(appendixLoci))))
DCJ <- as.data.frame(cbind(appendixLoci$Fis_DCJ, rep(x = "DCJ", nrow(appendixLoci))))

names(CARP) <- c("Fis", "Pop")
names(BR) <- c("Fis", "Pop")
names(SC) <- c("Fis", "Pop")
names(SM) <- c("Fis", "Pop")
names(DCJ) <- c("Fis", "Pop")

FisTable <- rbind(CARP, BR, SC, SM, DCJ)
FisTable$Fis <- as.numeric(as.character(FisTable$Fis))

p <- ggplot(FisTable, aes(x=Fis, fill = Pop)) +
  geom_histogram(binwidth = 0.02, color = "black") +
  facet_wrap(~Pop,nrow = 5)

p + ggtitle("") +
  xlab("Fis") + ylab("Locus Count") +
  theme_linedraw() +
  theme(strip.text.x = element_text(size = 14, angle = 0)) +
  theme(legend.position="none",
        text = element_text(size=14),
        axis.text.x = element_text(angle=0, hjust=1))
```


CARP
BR
SC
SM
DCJ
1500
1000
500
0
Locus Count
−1.0
−0.5
0.0
0.5
1.0
Fis

Minor allele frequencies per locus, per population

Distributions of minor allele frequencies for 11,970 SNP loci genotyped in sea lamprey at five spawning sites varied among populations.

```
CARP <- as.data.frame(cbind(appendixLoci$MAF_CARP, rep(x = "CARP", nrow(appendixLoci))))
BR <- as.data.frame(cbind(appendixLoci$MAF_BR, rep(x = "BR", nrow(appendixLoci))))
SC <- as.data.frame(cbind(appendixLoci$MAF_SC, rep(x = "SC", nrow(appendixLoci))))
SM <- as.data.frame(cbind(appendixLoci$MAF_SM, rep(x = "SM", nrow(appendixLoci))))
DCJ <- as.data.frame(cbind(appendixLoci$MAF_DCJ, rep(x = "DCJ", nrow(appendixLoci))))

names(CARP) <- c("MAF", "Pop")
names(BR) <- c("MAF", "Pop")
names(SC) <- c("MAF", "Pop")
names(SM) <- c("MAF", "Pop")
names(DCJ) <- c("MAF", "Pop")

MAFTable <- rbind(CARP, BR, SC, SM, DCJ)
MAFTable$MAF <- as.numeric(as.character(MAFTable$MAF))
MAFTable <- subset(MAFTable, MAF < 1 & MAF > 0)

p <- ggplot(MAFTable, aes(x=MAF, fill = Pop)) +
  geom_histogram(binwidth = 0.01, color = "black") +
  facet_wrap(~Pop,nrow = 5)

p + ggtitle("") +
  xlab("Minor Allele Frequency") + ylab("Locus Count") +
  theme_linedraw() +
  theme(strip.text.x = element_text(size = 14, angle = 0)) +
  theme(legend.position="none",
        text = element_text(size=14),
        axis.text.x = element_text(angle=0, hjust=1))
```


CARP
BR
SC
SM
DCJ
0
400
800
1200
Locus Count
0.0
0.1
0.2
0.3
0.4
0.5
Minor Allele Frequency

Outlier analysis

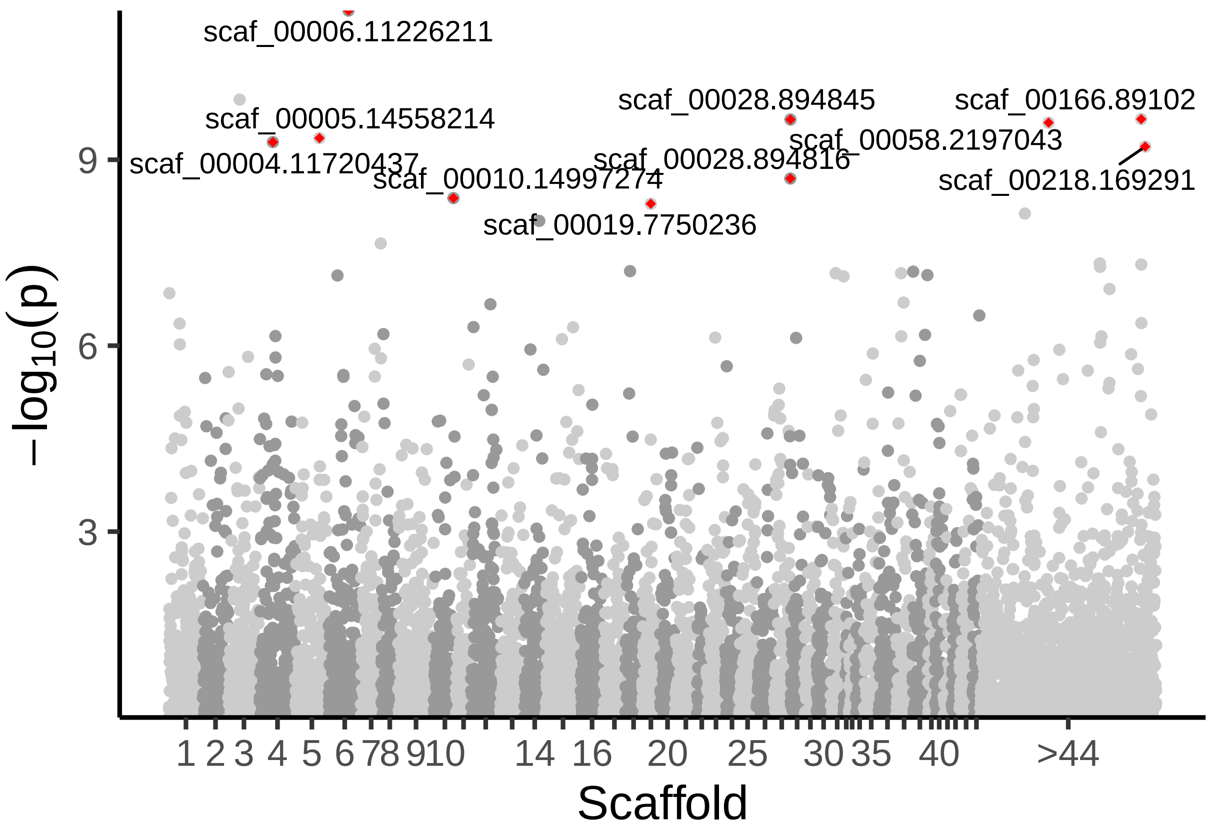
We performed an outlier analysis using OutFLANK (Whitlock & Lotterhos, 2015). A more comprehensively annotated version of this script is available at /analysis/outlierAnalysis.R

```
dat <- genotypeToSnpMatrix(allLoci.vcf, uncertain=FALSE)
dat1 <- as.data.frame(dat$genotypes@.Data)

write.csv(dat1, "../../tmp/data1temp.csv")
dat1 <- read.csv("../../tmp/data1temp.csv")

dat1[dat1 == 0] <- 9
dat1[dat1 == 1] <- 0
dat1[dat1 == 2] <- 1
dat1[dat1 == 3] <- 2

dat1$pop <- indPops$Pop

genotype <- dat1[, 2:(ncol(dat1)-1)]
ind <- paste("pop", dat1$pop)
locinames <- as.character(colnames(genotype))
FstDataFrame1 <- MakeDiploidFSTMat(genotype, locinames, ind)
```

```
## Calculating FSTs, may take a few minutes...
## [1] "10000 done of 12435"
```

```
out_trim <- OutFLANK(FstDataFrame1, NumberOfSamples=140, qthreshold = 0.1, Hmin = 0.1)

P1 <- pOutlierFinderChiSqNoCorr(FstDataFrame1, Fstbar = out_trim$FSTNoCorrbar,
                                dfInferred = out_trim$dfInferred, qthreshold = 0.1, Hmin=0.1)

substrRight <- function(x, n){
  substr(x, nchar(x)-n+1, nchar(x))
}

P1$Index <- as.integer(rownames(P1))
P1$label <- substr(P1$LocusName, 1, 100) # The names of loci
P1$label <- substr(P1$label, 1, nchar(P1$label)-4)
P1$transformed.p <- -log(P1$pvalues)
P1$scaffold <- as.integer(substr(P1$LocusName, 7, 10)) # The names of scaffolds
P1$scaffold[P1$scaffold > 44] <- 9999
P1$scaffold <- factor(P1$scaffold)

drops <- c("LocusName","He", "T1", "T2", "T1NoCorr", "T2NoCorr", "meanAlleleFreq", "pvalues",
           "pvaluesRightTail", "qvalues", "OutlierFlag")
plotting.df <- P1[ , !(names(P1) %in% drops)]

axis.df <- plotting.df %>% group_by(scaffold) %>%
  summarize(center = mean(Index)) %>%
  mutate(label = c("1", "2", "3", "4", "5", "6", "7", "8", "9", "10", " ", "", " ", "14", " ",
                   "16", " ", "", " ", "20", " ", "", " ", "", "25", "", " ", "", " ",
                   "30", " ", " ", " ", " ", "35", " ", " ", " ", " ", "40", " ", " ",
                   " ", " ", ">44"))

### Create Manhattan plot
```

```
ggplot(P1, aes(Index, transformed.p, label = label)) +
  geom_point(aes(color = P1$scaffold)) +
  ylab(expression(-log[10](p))) +
  scale_colour_manual(values = rep(c("gray80", "gray60"), 48), aesthetics = "color") +
  geom_text_repel(
    data = subset(P1, OutlierFlag == "TRUE")
  ) +
  geom_point(data = P1[P1$OutlierFlag == "TRUE", ], color = "red", shape = 18) +
  scale_x_continuous(label = axis.df$label, breaks= axis.df$center, name = "Scaffold") +
  scale_y_continuous(expand = c(0, 0) ) +
  theme_classic(base_size = 18) +
  theme(legend.position="none") +
  NULL
```



DAPC variable contributions, F_{ST} and q values estimated by OutFLANK, and distances, names 10 SNP loci with evidence of selection based on OutFLANK analysis.

SNP Scaffold	SNP Position	DAPC LD1 (%)	DAPC LD2 (%)	F_{ST}	OutFLANK q value
scaf_00004	11720437	0.985	0.694	0.220	0.053
scaf_00005	14558214	0.981	0.465	0.225	0.053
scaf_00006	11226211	0.957	0.343	0.263	0.041
scaf_00010	14997274	0.939	0.070	0.200	0.093
scaf_00019	7750236	0.934	0.053	0.201	0.093
scaf_00028	894816	0.988	0.782	0.210	0.077
scaf_00028	894845	0.987	0.703	0.232	0.053
scaf_00058	2197043	0.981	0.633	0.191	0.053
scaf_00166	89102	0.950	0.243	0.229	0.053
scaf_00218	169291	0.971	0.382	0.224	0.053

Figure 1: Outlier loci summary table