Vignette
FunciSNP: Functional Identification of SNPs with Phenotype by Coincidence with Chromatin Biofeatures

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February 13, 2012

Introduction

FunciSNP assist in identifying putative functional SNP from previously identified GWAS SNPs (tagSNP). Using information from the 1000 genomes database as well as known position of GWAS tagSNP currated for a particular trait or disease, FunciSNP integrates the two data along with sequence information provided by peaks identified from high-throughput sequencing. FunciSNP assumes user will provide peaks identified using any available ChIP peak algorithm, such as FindPeaks.

This vignette provides a 'HOW-TO' guide to setup and run FunciSNP on your machine. FunciSNP was developed with the idea that a user will have uninterupted high-speed internet access as well as a desktop machine with more than 4 multiple cores. If user is using a windows machine, multiple cores options will not work and thus total time to complete initial FunciSNP analysis will take longer than expected. Be sure you have uninterupted computing power when using a windows machine. If using a linux machine, please use 'screen' (see man screen for more information).

Using a 64bit Linux machine running 11.04 Ubuntu OS with 24G RAM and 8 cores connected to a academic high-speed internet port, the amount of time to complete 99 tagSNP across 20 different biofeatures took less than 30 min to complete. We anticipate about 2 hours to complete the same analysis using one core.

Load FunciSNP+other useful libraries

```
> #When package is offically posted in Bioconductor, uncomment next 2 lines.
> #source("http://bioconductor.org/biocLite.R")
```

```
> #biocLite("FunciSNP");
> ## Following two packages and options() are not required to run 'FunciSNP' but
> #will enhance the analysis experience.
> #library(setwidth); ## Automatically set the value of options("width") when the
> #terminal emulator is resized
> #library(colorout); ## colorize R output on terminal emulators
> options(width=80);
> ##FunciSNP library and other related libraries needed.
> library("org.Hs.eg.db");
> library("gplots");
> library("gtools");
> library("ggplot2");
> library("matlab");
> library(FunciSNP);
> package.version("FunciSNP");

[1] "0.1.8"
```

Identify FuncySNP using published GWAS SNPs and publicly available biological features (ENCODE ChIPseq peaks)

FuncySNP()

This section describes the main function of FunciSNP.

It will identify correlated SNPs which are in linkage disequilibrium (LD) to a known disease associated tagSNP. It will also determine if the correlated SNP in LD to the tagSNP overlaps a genomic biological feature. Correlated SNPs are directly imported from the current public release of the 1000 genomes database. 1000 genomes ftp servers available for the 1000 genomes public data: 1) National Center for Biotechnology Information (NCBI) ftp://ftp-trace.ncbi.nih.gov/1000genomes/; 2) European Bioinformatics Institute (EBI) ftp://ftp.1000genomes.ebi.ac.uk/vol1/.

Correlated SNPs in LD to a tagSNP and overlapping genomic biological features are known as putative functional SNPs (also defined as 'FuncySNP' elsewhere in the package.).

As an example, we collected SNPs identified by GWAS for Glioblastoma multiforme (GBM). In this example, GBM includes lower grade glioma, thus the use of 'glioma' to label all objects.

GWAS SNPs file should be in a tab or whitespace separated file. Three columns are required for each GWAS tagSNP. Position, rsID, population. Positon should be the exact postion for each rsID as determined by human genome build hg19 (Chromosome:Postion). rsID should contain a unique rsID as determined by the 1000 genomes database for each identified GWAS tagSNP. Population should be a three letter code to determine original ethnic population for which the associated tagSNP was identified. The three letter code should be either European (EUR), Asian (ASN), African (AFR), American (AMR), or All (ALL). List each tagSNP for multiple ethnic population.

```
> ## Full path to the example GWAS SNP regions file for Glioblastoma
> #  (collected from SNPedia on Jan 2012)
> glioma.snp <- file.path(system.file('extdata', package='FunciSNP'),
+ dir(system.file('extdata',package='FunciSNP'), pattern='.snp$'));
> gsnp <- read.delim(file=glioma.snp,sep=" ",header=FALSE);
> gsnp;
```

```
           V1        V2  V3
1 11:118477367  rs498872 EUR
2    5:1286516 rs2736100 ASN
3   9:22068652 rs4977756 EUR
4  20:62309839 rs6010620 EUR
```

Each biofeature used to identify correlated SNP should be in standard BED format. All biofeatures should be stored in one folder and should have file extension .bed. Here is an example of three different biofeatures used for the glioma example.

```
> #glioma.snp;
> ## Full path to the example biological features BED files
> #  derived from the ENCODE project for Glioblastoma U-87 cell lines.
> glioma.bio <- system.file('extdata',package='FunciSNP');
> list.files(glioma.bio, pattern='.bed$');
```

```
[1] "knownGene.TSS.hg19.bed" "TFBS_Nrsf_U87.bed"      "TFBS_Pol2_U87.bed"
```

```
> nrsf.filename <- list.files(glioma.bio, pattern='.bed$')[2];
> Nrsf <- read.delim(file=paste(glioma.bio, nrsf.filename,sep="/"), sep="\t",
+         header=FALSE);
> head(Nrsf);
```

```
   V1        V2        V3                      V4 V5 V6
1 chr5 178601706 178602140 Merged-chr5-178601923-1  0  +
2 chr5 178850156 178850592 Merged-chr5-178850374-1  0  +
3 chr5 179015119 179015553 Merged-chr5-179015336-1  0  +
4 chr7     23844     24636     Merged-chr7-24240-1  0  +
5 chr7     65601     66065     Merged-chr7-65833-1  0  +
6 chr7    128907    129421    Merged-chr7-129164-1  0  +
```

Following will take about 10 min to run.

```
> #glioma.bio;
> ## FunciSNP analysis, extracts correlated SNPs from the
> #  1000 genomes db ("ncbi" or "ebi") and finds overlaps between
> #  correlated SNP and biological features and then
> #  calculates LD (Rsquare, Dprime, distance, p-value).
> ## Depending on number of CPUs and internet connection, this step may take
> # some time. Please consider using a unix machine to access multiple cores.
> # glioma <- FuncySNP(snp.regions.file=glioma.snp,
> #           bio.features.loc = glioma.bio,
> #           bio.features.TSS=FALSE);
> # glioma;
> # summary(glioma);
```

If you decide not to run 'FuncySNP', you can call the results as follows. This was precompiled to provide user an example dataset to work with in this tutorial.

```
> data(glioma);
> glioma;

TagSNP List with  4  Tag SNPs and
 778 nearby,  potentially correlated SNPs, that overlap at least one biofeature
$`R squared: 0.1`
             Total R.squared.cuff.0.1 Percent
tagSNPs          4                  3   75.00
1kSNPs         778                 64    8.23
bio.features     3                  3  100.00

$`R squared: 0.5`
             Total R.squared.cuff.0.5 Percent
tagSNPs          4                  3   75.00
1kSNPs         778                 44    5.66
bio.features     2                  2  100.00

$`R squared: 0.9`
             Total R.squared.cuff.0.9 Percent
tagSNPs          4                  1   25.00
1kSNPs         778                 13    1.67
bio.features     2                  2  100.00

> summary(glioma);

TagSNP List with  4  Tag SNPs and
 778 nearby,  potentially correlated SNPs, that overlap at least one biofeature
Number of potentially correlated SNPs
overlapping at least x biofeatures, per Tag SNP at an R squared of
$`R squared: 0.1 in 4 Tag SNPs with a total of `
                       bio.1 bio.2
rs4977756                  3     0
rs498872                   9     2
rs6010620                 52     9
TOTAL # CORRELATED SNPS   64    11

$`R squared: 0.5 in 4 Tag SNPs with a total of `
                       bio.1 bio.2
rs4977756                  2     0
rs498872                   2     0
rs6010620                 40     6
TOTAL # CORRELATED SNPS   44     6

$`R squared: 0.9 in 2 Tag SNPs with a total of `
                       bio.1
rs6010620                 13
TOTAL # CORRELATED SNPS   13

> class(glioma);
```

```
[1] "TSList"
attr(,"package")
[1] "FunciSNP"
```

Annotating newly identified FuncySNPs

All known genomic features (exon, intron, 5'UTR, 3'UTR, promoter, lincRNA or in gene desert (intergentic)) are used to annotate each newly identified FuncySNP. Information described in this data.frame() is used for all summary plots, table, and to output results in BED format. This step should be completed after running FuncySNP().

```
> glioma.anno <- FunciSNPAnnotateSummary(glioma);
> class(glioma.anno);

[1] "data.frame"

> gl.anno <- glioma.anno;
> ## remove rownames for this example section.
> rownames(gl.anno) <- c(1:length(rownames(gl.anno)))
> dim(gl.anno);

[1] 862  28

> head(gl.anno); ##

  chromosome bio.feature.start bio.feature.end        bio.feature  corr.snp.id
1          5           1200710         1201809 knownGene.TSS.hg19 chr5:1200720
2          5           1200710         1201809 knownGene.TSS.hg19 chr5:1200766
3          5           1200710         1201809 knownGene.TSS.hg19 chr5:1200817
4          5           1200710         1201809 knownGene.TSS.hg19 chr5:1200946
5          5           1200710         1201809 knownGene.TSS.hg19 chr5:1200976
6          5           1200710         1201809 knownGene.TSS.hg19 chr5:1201033
  corr.snp.position tag.snp.id tag.snp.position   D.prime    R.squared p.value
1           1200720  rs2736100          1286516        NA           NA       1
2           1200766  rs2736100          1286516        NA           NA       1
3           1200817  rs2736100          1286516        NA           NA       1
4           1200946  rs2736100          1286516        NA           NA       1
5           1200976  rs2736100          1286516 1.0000000 0.0022585199       1
6           1201033  rs2736100          1286516 0.1795671 0.0004069606       1
  distance.from.tag population.count population nearest.lincRNA.ID
1            -85796              286        ASN     TCONS_00010241
2            -85750              286        ASN     TCONS_00010241
3            -85699              286        ASN     TCONS_00010241
4            -85570              286        ASN     TCONS_00010241
5            -85540              286        ASN     TCONS_00010241
6            -85483              286        ASN     TCONS_00010241
  nearest.lincRNA.distancetoFeature nearest.lincRNA.coverage
1                            -39302                upstream
2                            -39348                upstream
3                            -39399                upstream
```

```
4                           -39528                upstream
5                           -39558                upstream
6                           -39615                upstream
  nearest.TSS.GeneSymbol        nearest.TSS.refseq nearest.TSS.ensembl
1                SLC6A19 NM_001003841;NP_001003841     ENSG00000174358
2                SLC6A19 NM_001003841;NP_001003841     ENSG00000174358
3                SLC6A19 NM_001003841;NP_001003841     ENSG00000174358
4                SLC6A19 NM_001003841;NP_001003841     ENSG00000174358
5                SLC6A19 NM_001003841;NP_001003841     ENSG00000174358
6                SLC6A19 NM_001003841;NP_001003841     ENSG00000174358
  nearest.TSS.coverage nearest.TSS.distancetoFeature Promoter utr5 Exon Intron
1             upstream                          -990      YES   NO   NO     NO
2             upstream                          -944      YES   NO   NO     NO
3             upstream                          -893      YES   NO   NO     NO
4             upstream                          -764      YES   NO   NO     NO
5             upstream                          -734      YES   NO   NO     NO
6             upstream                          -677      YES   NO   NO     NO
  utr3 Intergenic
1   NO         NO
2   NO         NO
3   NO         NO
4   NO         NO
5   NO         NO
6   NO         NO
```

```
> names(gl.anno);
```

```
 [1] "chromosome"                        "bio.feature.start"
 [3] "bio.feature.end"                   "bio.feature"
 [5] "corr.snp.id"                       "corr.snp.position"
 [7] "tag.snp.id"                        "tag.snp.position"
 [9] "D.prime"                           "R.squared"
[11] "p.value"                           "distance.from.tag"
[13] "population.count"                  "population"
[15] "nearest.lincRNA.ID"                "nearest.lincRNA.distancetoFeature"
[17] "nearest.lincRNA.coverage"          "nearest.TSS.GeneSymbol"
[19] "nearest.TSS.refseq"                "nearest.TSS.ensembl"
[21] "nearest.TSS.coverage"              "nearest.TSS.distancetoFeature"
[23] "Promoter"                          "utr5"
[25] "Exon"                              "Intron"
[27] "utr3"                              "Intergenic"
```

```
> summary(gl.anno[,c(1:18,20:28)]);
```

```
  chromosome        bio.feature.start   bio.feature.end
 Length:862         Min.   :  1200710   Min.   :  1201809
 Class :character   1st Qu.: 62295044   1st Qu.: 62295926
 Mode  :character   Median : 62326155   Median : 62337392
                    Mean   : 65165595   Mean   : 65169512
                    3rd Qu.: 62374564   3rd Qu.: 62376020
                    Max.   :118531575   Max.   :118532674
```

```
            bio.feature           corr.snp.id  corr.snp.position
knownGene.TSS.hg19:372   chr11:118442863:  2   Min.   :  1200720
TFBS_Nrsf_U87     : 22   chr11:118443036:  2   1st Qu.: 62295889
TFBS_Pol2_U87     :468   chr11:118443046:  2   Median : 62327508
                         chr11:118478342:  2   Mean   : 65167605
                         chr20:62289690 :  2   3rd Qu.: 62375255
                         chr20:62289873 :  2   Max.   :118532636
                         (Other)        :850
    tag.snp.id  tag.snp.position       D.prime            R.squared
rs2736100: 96   Min.   :  1286516   Min.   :7.835e-04   Min.   :9.520e-08
rs4977756: 25   1st Qu.: 62309839   1st Qu.:9.338e-01   1st Qu.:7.765e-04
rs498872 :166   Median : 62309839   Median :1.000e+00   Median :4.501e-03
rs6010620:575   Mean   : 65163135   Mean   :8.995e-01   Mean   :1.258e-01
                3rd Qu.: 62309839   3rd Qu.:1.000e+00   3rd Qu.:2.804e-02
                Max.   :118477367   Max.   :1.000e+00   Max.   :9.776e-01
                                    NA's   :4.710e+02   NA's   :4.710e+02
   p.value           distance.from.tag population.count population
Min.   :2.115e-163   Min.   :-100000   Min.   :286.0    ASN: 96
1st Qu.: 1.000e+00   1st Qu.: -19966   1st Qu.:379.0    EUR:766
Median : 1.000e+00   Median :  13942   Median :379.0
Mean   : 7.989e-01   Mean   :   4470   Mean   :368.6
3rd Qu.: 1.000e+00   3rd Qu.:  25290   3rd Qu.:379.0
Max.   : 1.000e+00   Max.   :  67371   Max.   :379.0

     nearest.lincRNA.ID nearest.lincRNA.distancetoFeature
TCONS_00010241: 96      Min.   :-265183
TCONS_00015797: 25      1st Qu.: -92280
TCONS_00020001:166      Median :  59111
TCONS_00027984: 26      Mean   :   2073
TCONS_00028269:549      3rd Qu.:  73343
                        Max.   : 246019

nearest.lincRNA.coverage    nearest.TSS.GeneSymbol      nearest.TSS.ensembl
downstream:565           TNFRSF6B      :305        ENSG00000243509:305
inside    :  9           PHLDB1        : 86        ENSG00000019144: 86
upstream  :288           ZGPAT         : 68        ENSG00000197114: 68
                         RTEL1;TNFRSF6B: 37        ENSG00000229299: 59
                         SLC6A18       : 34        ENSG00000026036: 37
                         (Other)       :202        ENSG00000244977: 36
                         NA's          :130        (Other)        :271
nearest.TSS.coverage nearest.TSS.distancetoFeature Promoter   utr5
downstream:103       Min.   :-16454.0              NO :694   NO :825
inside    :311       1st Qu.: -3117.0              YES:168   YES: 37
upstream  :448       Median :   -76.0
                     Mean   :   890.4
                     3rd Qu.:  2305.8
                     Max.   : 28781.0

 Exon     Intron     utr3     Intergenic
```

```
 NO :776   NO :413   NO :702   NO :810
 YES: 86   YES:449   YES:160   YES: 52
```

```
> rm(gl.anno);
```

Summary table used to describe newly identified FuncySNPs

Using a specified Rsquare value (0-1) to subset the data, a table is generated which summarizes the total number of FuncySNPs, associated tagSNPs, and number of overlapping biofeatures. This will provide user a first look at the total number of available FuncySNP at a particular Rsquare cutoff.

```
> FunciSNPtable(glioma.anno, rsq=0.5);
```

```
             Total R.squared.cuff.0.5 Percent
tagSNPs          4                  3   75.00
1kSNPs         778                 44    5.66
bio.features     2                  2  100.00
```

If 'geneSum' is set to TRUE, a list of gene names is reported instead.

```
> FunciSNPtable(glioma.anno, rsq=0.5, geneSum=TRUE);
```

```
      Gene_Names
1         CDKN2B
2          LIME1
3         PHLDB1
4       SLC2A4RG
5       TNFRSF6B
6           TREH
7          ZGPAT
8 RTEL1;TNFRSF6B
```

Summary of correlated SNPs overlapping biofeatures

This function helps in determining the number of correlated SNPs overlapping a number of different biofeatures. This is similar to running 'summary(glioma)' above, except now you can specifically call the function and set a pre-determined 'rsq' value to subset the data and thereby obtain a more objective and informative result.

```
> FunciSNPsummaryOverlaps(glioma.anno)
```

```
                        bio.1 bio.2
rs2736100                  41     0
rs4977756                  12     0
rs498872                   59     3
rs6010620                 236    40
TOTAL # CORRELATED SNPS   348    43
```

Using a 'rsq' value, the output is subsetted to summarize the results with Rsquare values greater than or equal to 'rsq'.

```
> FunciSNPsummaryOverlaps(glioma.anno, rsq=0.5)

                        bio.1 bio.2
rs4977756                   2     0
rs498872                    2     0
rs6010620                  40     6
TOTAL # CORRELATED SNPS    44     6
```

Summary of correlated SNPs for a number of different tagSNPs

After running FunciSNPsummaryOverlaps(), the next question one would like to know is which correlated SNPs overlapping a number of different biofeatures for a number of associated tagSNP. Thus, in the example above, we have determined that we are interested in learning more about the correlated SNPs associated with 'rs6010620' and which overlap at least 2 different biofeatures.

```
> rs6010620 <- FunciSNPidsFromSummary(glioma.anno, tagsnpid="rs6010620",
+         num.features=2, rsq=0.5)
> summary(rs6010620);

  chromosome        bio.feature.start  bio.feature.end
 Length:12          Min.   :62326155   Min.   :62330994
 Class :character   1st Qu.:62329895   1st Qu.:62337392
 Mode  :character   Median :62354158   Median :62355398
                    Mean   :62351007   Mean   :62353861
                    3rd Qu.:62370211   3rd Qu.:62371310
                    Max.   :62371621   Max.   :62372970

            bio.feature    corr.snp.id corr.snp.position      tag.snp.id
 knownGene.TSS.hg19:6    rs1056441:2    Min.   :62330439   rs2736100: 0
 TFBS_Nrsf_U87     :0    rs1291209:2    1st Qu.:62330484   rs4977756: 0
 TFBS_Pol2_U87     :6    rs1295810:2    Median :62354704   rs498872 : 0
                         rs1741708:2    Mean   :62352184   rs6010620:12
                         rs6062498:2    3rd Qu.:62370732
                         rs6122159:2    Max.   :62372041
                         (Other)  :0
 tag.snp.position      D.prime          R.squared         p.value
 Min.   :62309839   Min.   :0.8380   Min.   :0.5073   Min.   :1.555e-127
 1st Qu.:62309839   1st Qu.:0.8979   1st Qu.:0.5365   1st Qu.:1.555e-127
 Median :62309839   Median :0.9204   Median :0.7588   Median :1.868e-117
 Mean   :62309839   Mean   :0.9039   Mean   :0.6967   Mean   : 1.046e-81
 3rd Qu.:62309839   3rd Qu.:0.9234   3rd Qu.:0.8092   3rd Qu.: 2.955e-89
 Max.   :62309839   Max.   :0.9234   Max.   :0.8092   Max.   : 6.274e-81

 distance.from.tag population.count population      nearest.lincRNA.ID
 Min.   :20600     Min.   :379      ASN: 0     TCONS_00010241: 0
 1st Qu.:20645     1st Qu.:379      EUR:12     TCONS_00015797: 0
 Median :44865     Median :379                 TCONS_00020001: 0
 Mean   :42345     Mean   :379                 TCONS_00027984: 0
 3rd Qu.:60893     3rd Qu.:379                 TCONS_00028269:12
```

```
 Max.   :62202     Max.   :379

 nearest.lincRNA.distancetoFeature nearest.lincRNA.coverage
 Min.   : 71755                    downstream:12
 1st Qu.: 71800                    inside    : 0
 Median : 96020                    upstream  : 0
 Mean   : 93500
 3rd Qu.:112048
 Max.   :113357

 nearest.TSS.GeneSymbol
 SLC2A4RG:6
 TNFRSF6B:4
 ZGPAT   :2
 ARCN1   :0
 ARFRP1  :0
 CDKN2B  :0
 (Other) :0

 NM_020062;NP_064446
 NM_003823;NP_003814
 NM_001083113;NM_001195653;NM_001195654;NM_032527;NM_181485;NP_001076582;NP_001182582;NP_0
 NM_001003841;NP_001003841
 NM_001037335;NM_033405;NP_001032412;NP_208384
 NM_001080441;NP_001073910
 (Other)
      nearest.TSS.ensembl nearest.TSS.coverage nearest.TSS.distancetoFeature
 ENSG00000125520:6        downstream:4         Min.   : 265
 ENSG00000243509:4        inside    :8         1st Qu.: 726
 ENSG00000197114:2        upstream  :0         Median :1764
 ENSG00000019144:0                             Mean   :1566
 ENSG00000026036:0                             3rd Qu.:2418
 ENSG00000049656:0                             Max.   :2463
 (Other)        :0
 Promoter  utr5     Exon    Intron   utr3   Intergenic
 NO :12   NO :12   NO :12   NO :8   NO :6   NO :10
 YES: 0   YES: 0   YES: 0   YES:4   YES:6   YES: 2

> dim(rs6010620);

[1] 12 28

> class(rs6010620);

[1] "data.frame"

> ## See FunciSNPbed to visualize this data in a genome browser.
```

Plot FunciSNP results

FunciSNPplot is a function developed to plot various types of plots to summarize and assist end-user in making informed discoveries of FunciSNP results. Plots can be stored in a folder for future reference. Most plots were created in publication standard.

The following example plots the distribution of the Rsquare values for each correlated SNP. We recommend attempting this plot before subsetting any data by a specified rsq value. The distribution helps to identify a specific Rsquare value that will provide the most informative discovery.

```
> pdf("glioma_dist.pdf")
> FunciSNPplot(glioma.anno)
> dev.off()

null device
          1
```

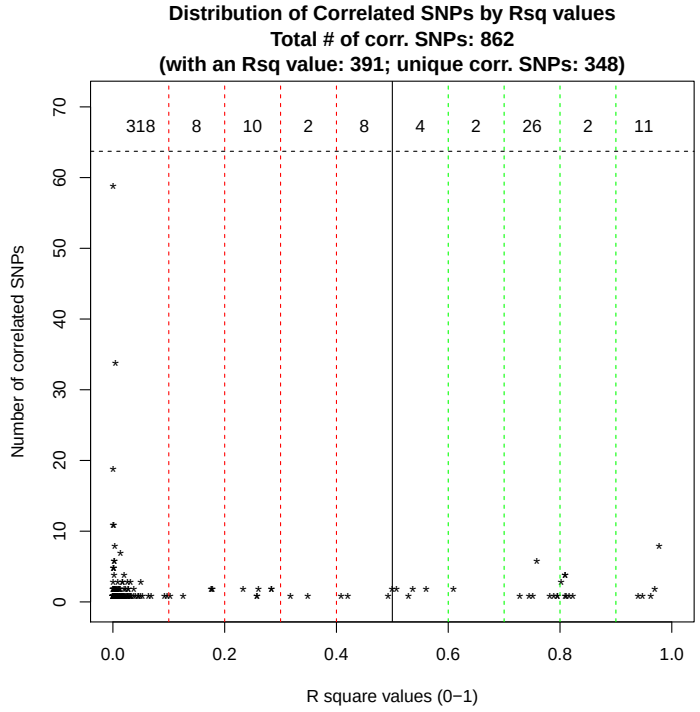

Figure 1: Distribution of Rsquare values of all Correlated SNPs. Each marked bin contains the total number of correlated SNPs. The sum of all the counts would total the number of correlated SNPs.

Using splitbysnp argument, the same type of plot as above (Figure **??**) is generated, however the total number of correlated SNPs are divided by the associated tagSNP.

```
> FunciSNPplot(glioma.anno, splitbysnp=TRUE)
> ggsave("glioma_dist_bysnp.pdf")
```

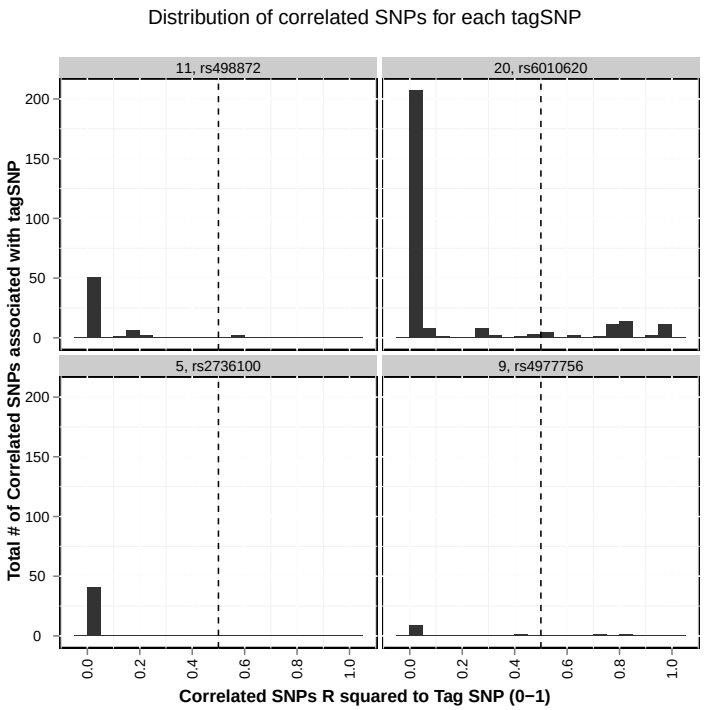

Figure 2: Distribution of Rsquare values of all Correlated SNPs divided by the tagSNP and it's location.

Using genomicSum argument set to TRUE will output the overall genomic distribution of the newly identified correlated SNPs. Using 'rsq' value, the plot is divided into all correlated SNPs vs subset. This type of plot informs the relative enrichment for genomic features.

```
> pdf("glioma_genomic_sum_rcut.pdf")
> FunciSNPplot(glioma.anno, rsq=0.5, genomicSum=TRUE, save=FALSE)
> dev.off()

X11cairo
       2
```

'tagSummary' argument is unique in that it will automatically save all plots in a specific folder. This is done because this function will generate a summary plot for each biofeature. The first plot (Figure **??**) is a scatter plot showing the relationship between Rsquare and Distance to tagSNP for each FuncySNP. The second plot (Figure **??**) is a histogram distribution of total number of correlated SNPs at each Rsquare value. This plot is similar to Figure **??**, except it is further divided by biofeature. Each set of plot is further divided by tagSNP to help

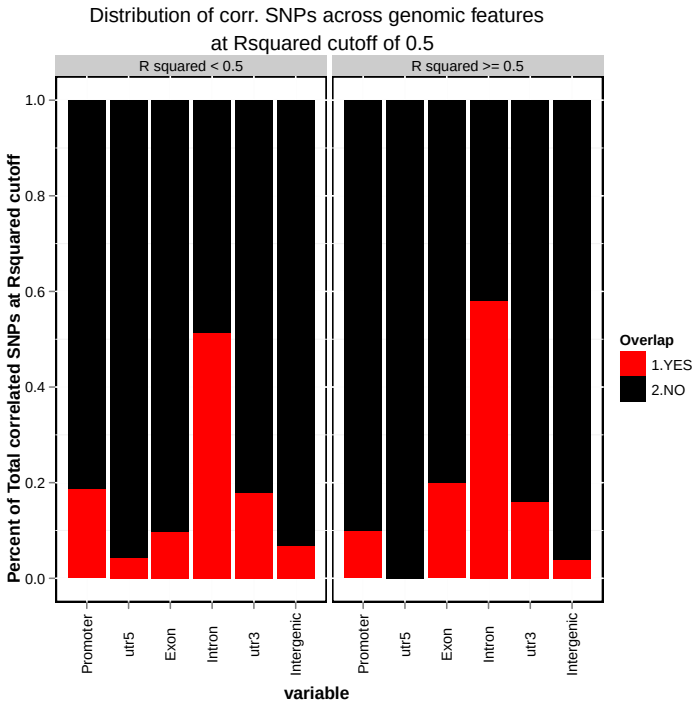

Figure 3: Stacked bar chart summarizing all correlated SNPs for each of the identified genomie features: exon, intron, 5UTR, 3UTR, promoter, lincRNA or in gene desert. Rsquare cutoff at 0.5. This plot is most informative if used with a rsq value.

identify locus with the most identifiable FuncySNP. This argument is best used in conjunction with a 'rsq' value.

```
> ## Following will output a series of plots for each biofeature at rsq=0.5
> FunciSNPplot(glioma.anno, tagSummary=TRUE, rsq=0.5)

Finished plotting  1 / 3
Finished plotting  2 / 3
Finished plotting  3 / 3
```

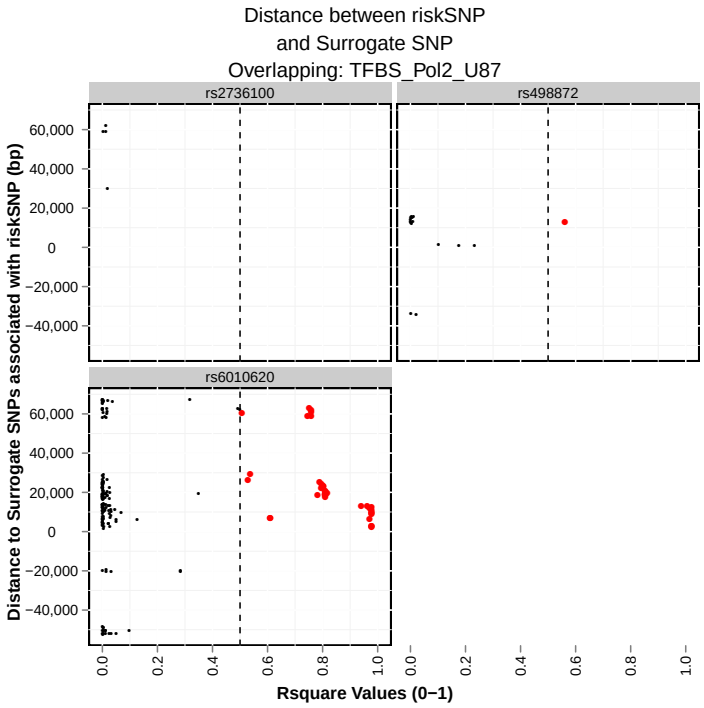

Figure 4: Scatter plot showing the relationship between Rsquare and Distance to tagSNP for each FuncySNP

Output results in BED format - visualize results

Finally, after evaluating all results using the above tables and plots functions, a unique pattern emerges that helps identifies a unique cluster of tagSNP and biofeature that can identify a set of FuncySNPs. To better visualize and to get a better perspective of the location of each newly identified FuncySNP, the results can be outputted using FunciSNPbed.

FunciSNPbed outputs a unique BED file which can be used to view in any genomic browser compatible with BED formats. To learn more about BED formats, see UCSC Genome Browser FAQ (http://genome.ucsc.edu/FAQ/FAQformat). Each tagSNP which is in LD to a corresponding FuncySNP overlapping at least one biofeature is colored black, while the FuncySNP is colored red. The initial position is provided by the first tagSNP and the first linked FuncySNP. We recommend using UCSC genome browser to view your BED files. This is useful so you can view all public and private tracks in relation to FunciSNP results.

```
> ## will output to current working directory.
> FunciSNPbed(glioma.anno, rsq=0.5);

Total corSNP (RED):  44
Total tagSNP (BLK):  3

> # FunciSNPbed(rs6010620, rsq=0.5);
```

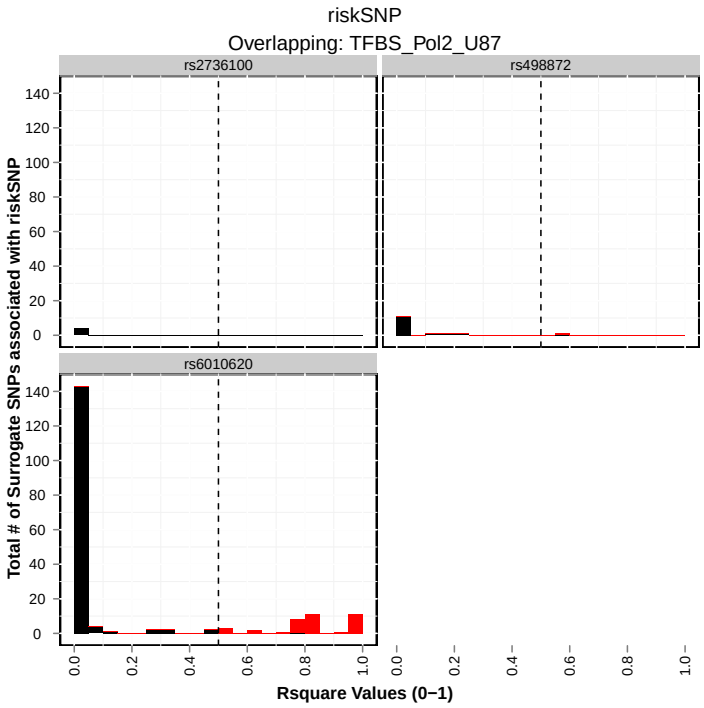

Figure 5: Histogram distribution of number of correlated SNPs at each Rsquare value

Questions or comments, please contact Simon G. Coetzee (scoetzee NEAR gmail POINT com) or Houtan Noushmehr (houtana NEAR gmail POINT com).

```
> sessionInfo()

R version 2.14.1 (2011-12-22)
Platform: x86_64-pc-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=C                 LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
 [1] tools     splines   grid      stats     graphics  grDevices utils
 [8] datasets  methods   base

other attached packages:
 [1] FunciSNP_0.1.8
```

```
 [2] VariantAnnotation_1.0.5
 [3] TxDb.Hsapiens.UCSC.hg19.knownGene_2.6.2
 [4] GenomicFeatures_1.6.7
 [5] ChIPpeakAnno_2.2.0
 [6] limma_3.10.2
 [7] GO.db_2.6.1
 [8] BSgenome.Ecoli.NCBI.20080805_1.3.17
 [9] BSgenome_1.22.0
[10] multtest_2.10.0
[11] biomaRt_2.10.0
[12] GGtools_4.0.0
[13] ff_2.2-5
[14] bit_1.1-8
[15] annotate_1.32.1
[16] GGBase_3.14.0
[17] genefilter_1.36.0
[18] snpStats_1.4.1
[19] Matrix_1.0-3
[20] lattice_0.20-0
[21] survival_2.36-10
[22] rtracklayer_1.14.4
[23] RCurl_1.9-5
[24] Rsamtools_1.6.3
[25] Biostrings_2.22.0
[26] GenomicRanges_1.6.6
[27] IRanges_1.12.5
[28] matlab_0.8.9
[29] ggplot2_0.8.9
[30] proto_0.3-9.2
[31] reshape_0.8.4
[32] plyr_1.7.1
[33] gplots_2.10.1
[34] KernSmooth_2.23-7
[35] caTools_1.12
[36] bitops_1.0-4.1
[37] gdata_2.8.2
[38] gtools_2.6.2
[39] org.Hs.eg.db_2.6.4
[40] RSQLite_0.11.1
[41] DBI_0.2-5
[42] AnnotationDbi_1.16.11
[43] Biobase_2.14.0

loaded via a namespace (and not attached):
[1] digest_0.5.1    MASS_7.3-16     parallel_2.14.1 XML_3.9-4
[5] xtable_1.6-0    zlibbioc_1.0.0
```