traseR: TRait-Associated SNP EnRichment analyses

Li Chen, Zhaohui S.Qin

Department of Biostatistics and Bioinformatics
Emory University
Atlanta, GA 303022

li.chen@emory.edu,zhaohui.qin@emory.edu

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Contents

Abstract

This vignette introduces the use of traseR (**TR**ait-**A**ssociated SNP**E**n**R**ichment analyses, which is designed to provide quantitative assessment whether a selected genomic interval(s) is likely to be functionally connected with certain traits or diseases. traseR consists of several modules, all written in R, to perform hypothesis testing, exploration and visualization of trait-associated SNPs(taSNPs). It also assembles the up-to-date taSNPs from dbGaP and NHGRI, SNPs from 1000 Genome Project CEU population with linkage disequilibrium greater than 0.8 within 100 kb of taSNPs, and all SNPs of CEU population from 1000 Genome project into its built-in database, which could be directly loaded when performing analyses.

1 Introduction

Genome-wide association study (GWAS) have successfully identified many sequence variants that are significantly associated with common diseases and traits. Tens of thousands of such trait-associated SNPs have already been cataloged which we believe are great resources for genomic research. However, no tools existing utilizes those resources in a comprehensive and convenient way. In this study, we show the collection of taSNPs can be exploited to indicate whether a query genomic interval(s) is likely to be functionally connected with certain traits or diseases. A R Bioconductor package named traseR has been developed to carry out such analyses.

2 Data collection

One great feature of traseR is the built-in database that collects various public SNP resources. Common public SNP databases include Association Result Browser and 1000 Genome Project. We briefly introduce the procedures to process those public available SNP resources

2.1 Obtain taSNPs

Association Results Browser (http://www.ncbi.nlm.nih.gov/projects/gapplusprev/sgap_plus.htm) combines identified taSNPs from dbGaP and NHGRI, which together provide 44,078 SNP-trait associations, 48,936 SNP-trait class associations, 30,553 unique taSNPs, 573 unique traits and 33 unique trait classes. This resource has been built into GRanges object taSNP and could be loaded into R console by typing `data(taSNP)`.

traseR need to specify the collection of trait-associated SNPs in particular format before we carry out enrichment analyses. The format starts with the columns,

1. Trait: Description of disease/trait examined in the study
2. Trait_Class: Trait class which is formed based on the phenotype tree. Close traits are grouped together to form one class.
3. SNP_ID: SNP rs number
4. p.value: GWAS reported p-values
5. seqnames: Chromosome number associated with rs number
6. ranges: Chromosomal position, in base pairs, associated with rs number
7. Context: SNP functional class

8. GENE_NAME: Genes reported to be associated with SNPs
9. GENE_START: Chromosome start position of genes
10. GENE_END: Chromosome end position of genes
11. GENE_STRAND: Chromosome strand associated with SNPs

Currently, the `traseR` package automatically synchronize trait-associated SNPs from Association Results Browser, which collects up-to-date GWAS results from dbGaP NHGRI GWAS catalog.

2.2 Obtain linkage disequilibrium taSNPs from 1000 Genome Project

We first download CEU vcf files from (ftp://share.sph.umich.edu/1000genomes/fullProject/2012.03.14/) that contain all sequence variants information. The followed two steps are used to identify linkage disequilibrium SNPs >0.8 and located within 100kb of taSNPs. Firstly, we use `vcftools` to convert the vcf file format to `PLINK` format. Then we use `PLINK` to call the LD taSNPs by specifying options that limit the linkage disequilibrium SNPs >0.8 (–ld-window-r2 0.8) and within 100kb of taSNP (–ld-window-kb 100). The detailed commands are listed below,

```
vcftools -vcf vcf.file -out plink.file -plink plink -file plink.file -r2 -inter-chr -ld-snp-list
snps.txt -ld-window-r2 0.8 -ld-window-kb 100 -out output.file -noweb
```

Finally, we have 90,700 SNP-trait associations and 78,247 unique linkage disequilibrium trait-associated SNP. We also build linkage disequilibrium taSNP into another GRanges object `taSNPLD`, which could be loaded into R console by typing `data(taSNPLD)`.

The format of `taSNPLD` is,

1. seqnames: Chromosome number associated with rs number
2. SNP_ID: SNP rs number
3. ranges: Chromosomal position, in base pairs, associated with rs number
4. Trait: Description of disease/trait examined in the study
5. Trait_Class: Trait class which is formed based on the phenotype tree. Close traits are grouped together to form one class.

2.3 Obtain background SNPs from 1000 Genome Project

We use the command `plink -file plink.file -freq -out chr` to retrieve all SNPs with corresponding MAF (minor allele frequency) from the CEU vcf files downloaded. There are totally 6,571,512 SNPs (MAF>0.05) excluding variants on Y chromosome. Those SNPs could serve as background in hypothesis testing. We build those SNPs into the built-in GRanges subject `CEU` into the package.

The format of `CEU` is,

1. seqnames: Chromosome number associated with rs number
2. SNP_ID: SNP rs number
3. ranges: Chromosomal position, in base pairs, associated with rs number

3 Using traseR

To assess the enrichment level of trait-associated SNPs in given genomic interval(s) using traseR, one needs to follow the simple steps below.

1. Prepare the genomic intervals in R object of either data frame format with column names chr,start,end or GRanges object
2. Query a given a set of genomic interval(s) against all the taSNPs in the collection, perform statistical analyses
3. Explore genes/SNPs of particular interest

3.1 Background selection

3.1.1 Whole genome

The assumption is each base could be possibly be the taSNP. Based on the assumption, with the number of taSNPs inside and outside the genomic interval(s), the number of bases inside and outside of the genomic interval(s), we could classify all bases based on the fact that one base is taSNP or not and in genomic intervals or not.

3.1.2 All SNPs

The assumption is each SNP could possibly be the taSNP. Based on the assumption, with the number of taSNPs inside and outside the genomic interval(s), the non-taSNPs inside and outside of the genomic interval(s), we could classify all SNPs based on the fact that one SNP is taSNP or not and in genomic intervals or not.

3.2 Hypothesis testing

traseR provides differential hypothesis testing methods in core function traseR, together with other functions for exploring and visualizing the results. The genomic interval(s) could be a data frame with three columns as chr(chromosome), start(genomic start position) and end(genomic end position) or a GRanges object. traseR offers either including LD SNPs or excluding LD SNPs as the taSNPs and either using the whole genome or all SNPs as the background for hypothesis testing.
If using whole genome as background, the command line is:

```
> x=traseR(snpdb=taSNP,region=Tcell)
> print(x)
```

If including the LD SNPs, the command line is:

```
> x=traseR(snpdb=taSNPLD,region=Tcell)
> print(x)
```

If using all SNPs as background, the command line is:

```
> x=traseR(snpdb=taSNP,region=Tcell,snpdb.bg=CEU)
```

For the above commands, `region` is the data frame; `snpdb` is taSNPs or including LD SNPs; `snpdb.bg` is background SNPs; If `rankby` is set as "pvalue", all traits will be sorted by p-value in increasing order; if`rankby` is set as "odds.ratio", all traits will be sorted by odds ratio in decreasing order. There are four options for `test.method` including "binomial", "chisq", "fisher", and "nonparametric"to perform binomial test, χ^2 test, Fisher's exact test and nonparametric respectively. If `alternative` is set to "greater", `traseR` will perform hypothesis testing on whether genomic intervals are enriched of taSNPs than the background; If `alternative` is set to "less",`traseR` will perform hypothesis testing on whether genomic intervals are depleted of taSNPs than the background.

3.2.1 χ^2 test and Fisher's exact test

Based on which background we choose, we could construct the 2 by 2 contingency table. then, we could perform χ^2 test on the table to assess the difference of proportions of taSNPs inside and outside of genomic intervals(s). We could also assume taSNPs inside genomic intervals follows hypergeometric distribution and calculate p-value directly using Fisher's exact test.

3.2.2 Binomial test

The assumption is the probability of observing a single base/SNP being a taSNP is the same inside and outside of genomic intervals. The probability of observing a single base/SNPs being a taSNP in genomic intervals could be estimated by using total number of taSNPs divided by the genome size/number of all SNPs. Then corresponding p-value could be calculated directly by Binomial test.

3.2.3 Nonparametric Test

Instead of imposing any assumption, the matched genomic interval(s) are generated by permuting the genomic intervals randomly N times and overlap with taSNPs in each time. Then we could calculate the empirical p-value directly by counting how many taSNP hits larger/smaller than the observed taSNP hits.

4 Choose appropriate statistical test method

Depending on the characteristics of the test statistics, we suggest to choose appropriate statistical test method under different scenarios,

- χ^2 test: the numbers in the contingency table is fairly large and balanced
- Fisher's exact test: the numbers of the contingency table is relatively small, e.g. no more than 20
- Nonparametric test: the number of query genomic intervals are small, e.g. no more than 1000
- Binomial test: default test method, not limited by sample size, distribution assumption and computational time

4.1 Example

To further illustrate the usage of traseR R package, we download H3K4me1 peak regions in peripheral blood T cell from Roadmap Epigenomics. Those peak regions are deemed the genomic intervals. Since the degree of enrichment level is measured by p-value, we could rank traits/trait classes based on p-value in an increasing order. We choose Binomial test are the default option for test.method, use whole genome as background and over-enrichment as hypothesis testing direction.

```
> library(traseR)
> data(taSNP)
> data(Tcell)
> x=traseR(taSNP,Tcell)
> print(x)
```

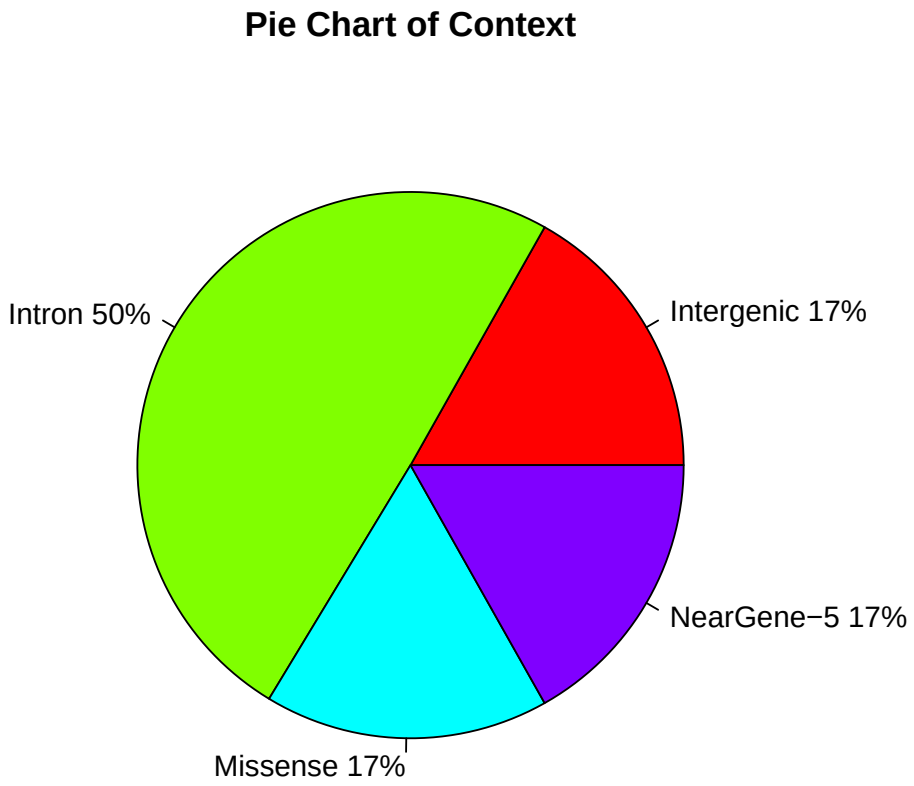
```
  Trait       p.value odds.ratio taSNP.hits taSNP.num
1   All 3.788373e-233   2.134717       2625     30553
                           Trait      p.value      q.value odds.ratio taSNP.hits
67                Behcet Syndrome 4.400406e-23 2.521433e-20   6.306579         59
172     Diabetes Mellitus, Type 1 1.704981e-11 4.884769e-09   5.045263         33
340 Lupus Erythematosus, Systemic 6.159346e-09 1.176435e-06   3.902195         32
49           Arthritis, Rheumatoid 1.442123e-07 2.065841e-05   5.126637         20
379             Multiple Sclerosis 1.644125e-05 1.884167e-03   2.905210         26
62             Autoimmune Diseases 5.201529e-05 4.967461e-03  15.892575          6
    taSNP.num
67        274
172       185
340       223
49        112
379       236
62         15
                          Trait_Class      p.value      q.value odds.ratio taSNP.hits
17               Immune System Diseases 3.729169e-35 1.143835e-33   3.658860        155
31 Skin and Connective Tissue Diseases 6.932335e-35 1.143835e-33   3.916319        142
32              Stomatognathic Diseases 1.041455e-22 1.145601e-21   5.675922         63
14                         Eye Diseases 3.479491e-18 2.870580e-17   3.313308         87
11            Digestive System Diseases 4.362324e-14 2.879134e-13   3.040672         74
7               Cardiovascular Diseases 3.008551e-11 1.654703e-10   1.602762        253
13            Endocrine System Diseases 6.933337e-09 3.268573e-08   2.068149         89
24  Nutritional and Metabolic Diseases 4.763509e-08 1.964948e-07   2.068673         79
21             Musculoskeletal Diseases 3.118359e-05 1.143398e-04   2.716680         27
23              Nervous System Diseases 5.549981e-05 1.831494e-04   1.495744        122
16         Hemic and Lymphatic Diseases 1.649261e-04 4.947782e-04   3.596622         15
22                           Neoplasms 3.372076e-04 9.273210e-04   1.580636         76
```

```
30          Respiratory Tract Diseases 6.507770e-04 1.651972e-03   2.121839         29
   taSNP.num
17      1122
31       970
32       318
14       689
11       633
7       3850
13      1076
24       956
21       260
23      1988
16       115
22      1181
30       349
```

4.2 Exploratory and visualization functions

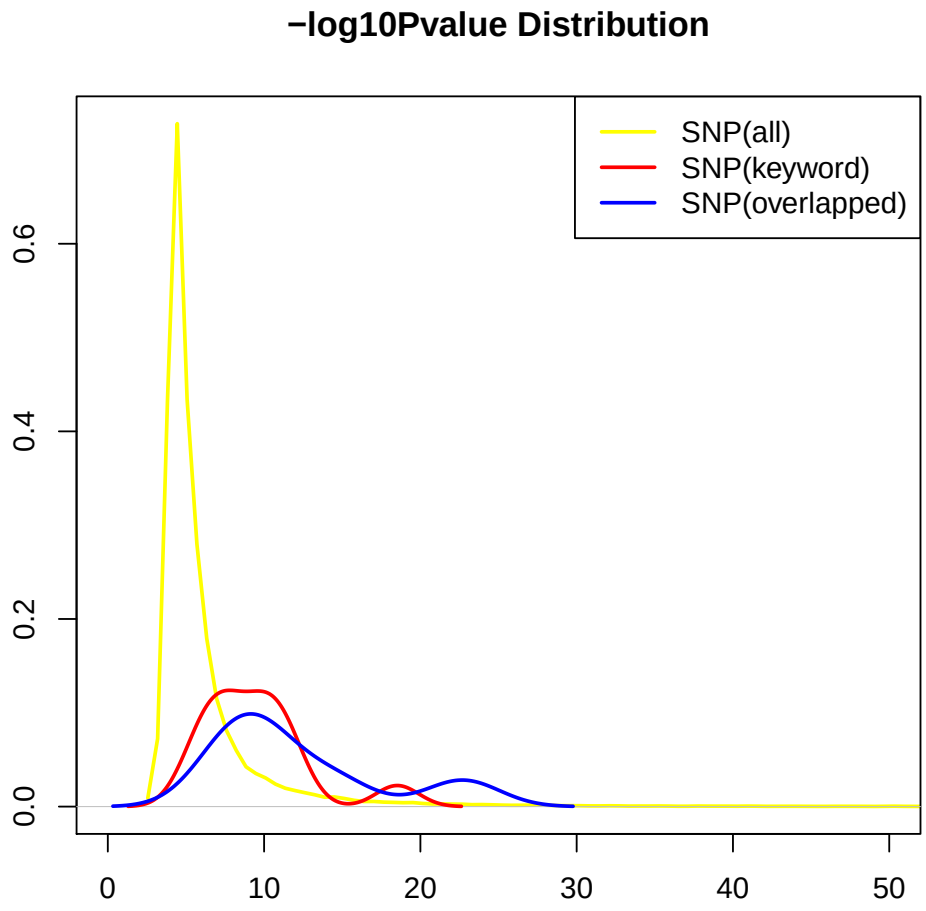
Plot the distribution of SNP functional class

```
> plotContext(snpdb=taSNP,region=Tcell,keyword="Autoimmune")
```



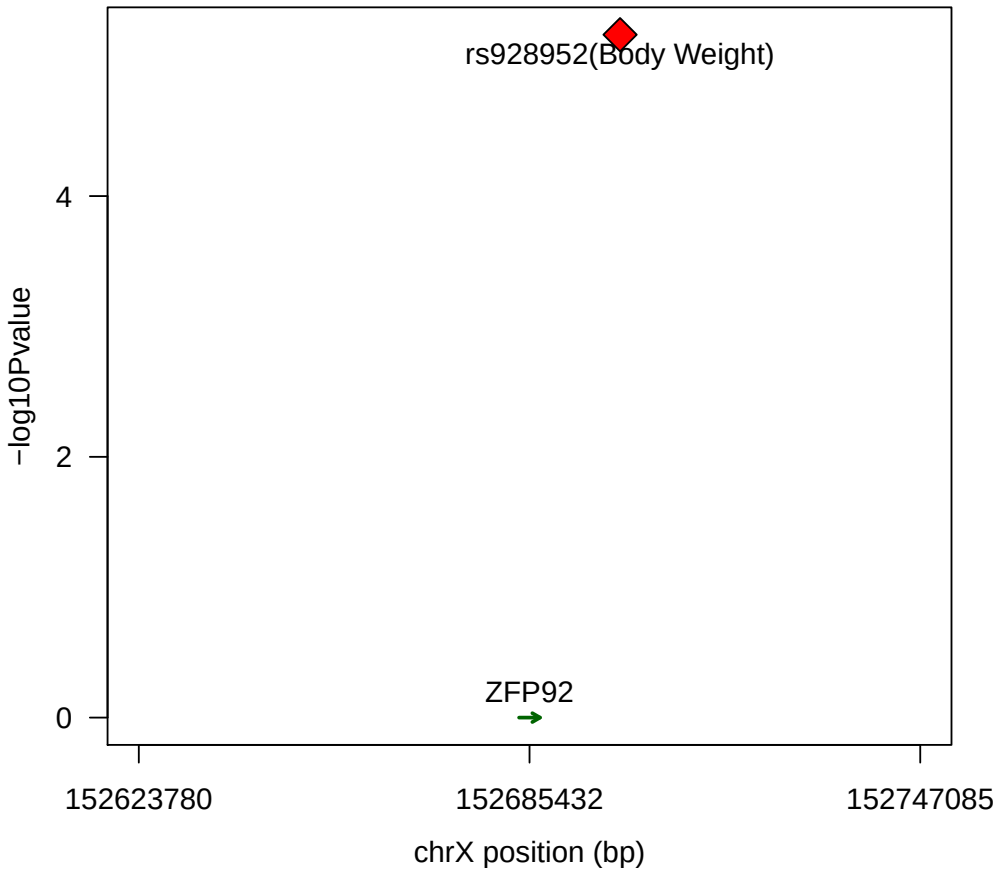
Plot the distribution of p-value of trait-associated SNPs

```
> plotPvalue(snpdb=taSNP,region=Tcell,keyword="autoimmune",plot.type="densityplot")
```



Plot SNPs or genes given genomic interval

```
> plotInterval(snpdb=taSNP,data.frame(chr="chrX",start=152633780,end=152737085))
```



Query trait-associated SNPs by key word,

```
> x=queryKeyword(snpdb=taSNP,region=Tcell,keyword="autoimmune",returnby="SNP")
> head(x)
```

```
         SNP_ID   Chr   Position Trait.num          Trait.name
4343 rs11203203 chr21   43836186         1 Autoimmune Diseases
4341  rs1876518  chr2   65608909         1 Autoimmune Diseases
4348  rs1953126  chr9  123640500         1 Autoimmune Diseases
4342  rs2298428 chr22   21982892         1 Autoimmune Diseases
4345  rs7579944  chr2   30445026         1 Autoimmune Diseases
4338   rs864537  chr1  167411384         1 Autoimmune Diseases
```

Query trait-associated SNPs by gene name,

```
> x=queryGene(snpdb=taSNP,genes=c("AGRN","UBE2J2","SSU72"))
> x
```

```
GRanges object with 3 ranges and 5 metadata columns:
      seqnames             ranges strand | GENE_NAME Trait.num          Trait.name
         <Rle>          <IRanges>  <Rle> |  <factor> <integer>            <factor>
  [1]     chr1 [ 955502,  991491]      + |      AGRN         1     Body Mass Index
  [2]     chr1 [1477052, 1510261]      - |     SSU72         1             Glucose
  [3]     chr1 [1189291, 1209233]      - |    UBE2J2         1 Waist Circumference
      taSNP.num taSNP.name
      <integer>   <factor>
  [1]         1  rs3934834
  [2]         1   rs880051
  [3]         1 rs11804831
  -------
  seqinfo: 23 sequences from an unspecified genome; no seqlengths
```

Query trait-associated SNPs by SNP name,

```
> x=querySNP(snpdb=taSNP,snpid=c("rs3766178","rs880051"))
> x
```

```
GRanges object with 2 ranges and 9 metadata columns:
        seqnames             ranges strand |       Trait      SNP_ID   p.value
           <Rle>          <IRanges>  <Rle> | <character> <character> <numeric>
  42234     chr1 [1478180, 1478180]      * |     Glucose   rs3766178  3.26e-05
  42127     chr1 [1493727, 1493727]      * |     Glucose    rs880051  6.44e-05
            Context   GENE_NAME GENE_START  GENE_END GENE_STRAND
        <character> <character>  <integer> <integer> <character>
  42234      Intron       SSU72    1477052   1510261           -
  42127      Intron       SSU72    1477052   1510261           -
                        Trait_Class
                        <character>
  42234 Chemicals and Drugs Category
  42127 Chemicals and Drugs Category
  -------
  seqinfo: 23 sequences from an unspecified genome; no seqlengths
```

5 Conclusion

traseR provides methods to assess the enrichment level of taSNPs in a given sets of genomic intervals. Moreover, it provides other functionalities to explore and visualize the results.

6 Session Info

```
> sessionInfo()

R version 3.3.1 (2016-06-21)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 16.04.1 LTS

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

attached base packages:
[1] parallel  stats4    stats     graphics  grDevices utils     datasets  methods
[9] base

other attached packages:
 [1] traseR_1.4.0                    BSgenome.Hsapiens.UCSC.hg19_1.4.0
 [3] BSgenome_1.42.0                 rtracklayer_1.34.0
 [5] Biostrings_2.42.0               XVector_0.14.0
 [7] GenomicRanges_1.26.0            GenomeInfoDb_1.10.0
 [9] IRanges_2.8.0                   S4Vectors_0.12.0
[11] BiocGenerics_0.20.0

loaded via a namespace (and not attached):
 [1] lattice_0.20-34            XML_3.98-1.4               Rsamtools_1.26.0
 [4] GenomicAlignments_1.10.0   bitops_1.0-6               grid_3.3.1
 [7] zlibbioc_1.20.0            Matrix_1.2-7.1             BiocStyle_2.2.0
[10] BiocParallel_1.8.0         tools_3.3.1                Biobase_2.34.0
[13] RCurl_1.95-4.8             SummarizedExperiment_1.4.0
```

References

[1] Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L et al (2010). The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acid Research*, **42**, D1001-1006.

[2] Roadmap Epigenomics C, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J et al. (2015). Integrative analysis of 111 reference human epigenomes *Nature*, **7539**, 317-330

[3] http://www.ncbi.nlm.nih.gov/projects/gapplusprev/sgap_plus.htm *Association Results Browser*

[4] ftp://share.sph.umich.edu/1000genomes/fullProject/2012.03.14/ *1000Genome EUR*