

# traseR: TRait-Associated SNP EnRichment analyses

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### Abstract

This vignette introduces the use of traseR (TRait-Associated SNPEnRichment 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 HapMap project into its built-in database, which could be directly loaded when performing analyses.

## 1 Introduction

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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 is a great resource 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 selected 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

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One great feature of traseR is the built-in database that collects various public SNP resources. Common public SNP databases include Association Result Browser, HapMap and 1000 Genome. 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](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, 30,553 unique taSNPs and 573 unique traits. This resource has been built into GRanges object taSNPDB and could be loaded into R console by typing `data(taSNPDB)`.

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. SNP\_ID: SNP rs number
3. p.value: GWAS reported p-values
4. seqnames: Chromosome number associated with rs number
5. ranges: Chromosomal position, in base pairs, associated with rs number
6. Context: SNP functional class
7. GENE\_NAME: Genes reported to be associated with SNPs
8. GENE\_START: Chromosome start position of genes
9. GENE\_END: Chromosome end position of genes
10. 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

We first download all vcf files from (<ftp://share.sph.umich.edu/1000genomes/fullProject/2012.03.14/>) that contains all genetic variants information. Then, two steps are used to find 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 90700 SNP-trait associations and 78247 unique linkage disequilibrium trait-associated SNP. We also build linkage disequilibrium taSNP into another GRanges object taSNPLDDB, which could be loaded into R console by typing `data(taSNPLDDB)`.

The format of taSNPLDDB 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

## 2.3 Obtain SNPs from HapMap

We download all genotype files in CEU population from the site, (<http://hapmap.ncbi.nlm.nih.gov/downloads/genotypes/latest/forward/non-redundant/>) The site contains merged phase I+II and III HapMap genotype files. We treat all alleles in the genotype files as SNPs for CEU population, which are expressed in the forward strand relative to the reference genome sequence. There are totally 4,029,840 variants excluding variants on Y chromosome, which could serve as background in hypothesis testing. Since all genotype files are in text format, we simply include all SNPs in auto chromosome and chromosome X into package built-in GRanges subject CEU.

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

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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 data frame format with column names `chr`, `start`, `end`

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 a base is taSNP or not and a base is in genomic intervals or not.

#### 3.1.2 All SNPs

The assumption is each SNP could be 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 a SNP is taSNP or not and a SNP is 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). `traseR` also offers 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=taSNPDB,region=Tcell)
> print(x)
```

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

```
> x=traseR(snpdb=taSNPLDDB,region=Tcell)
```

For the above commands, `region` is the data frame; `snpdb` is taSNPs; `snpdb.bg` is a background non-taSNPs; 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,  $\chi^2$  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 $\chi^2$ and Fisher's exact test

Based on which background we choose, we could construct the 2 by 2 contingency table. then, we could perform  $\chi^2$  test on the table to assess the goodness of fit of the distribution of taSNPs inside and outside of genomic intervals(s).

We could also assume taSNPs in genomic intervals follows hypergeometric distribution and could calculate the p-value by 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/All SNPs. Then corresponding p-value could be calculated by using binomial test.

### 3.2.3 Nonparametric Test

Instead of enforcing any assumption, the control genomic interval(s) are generated by shuffling 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.

## 3.3 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 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(taSNPDB)
> data(Tcell)
> x=traseR(taSNPDB,Tcell)
> print(x)
```

	Trait	p.value	odds.ratio	taSNP.hits	taSNP.num
1	All	2.771864e-48	1.460704	1846	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

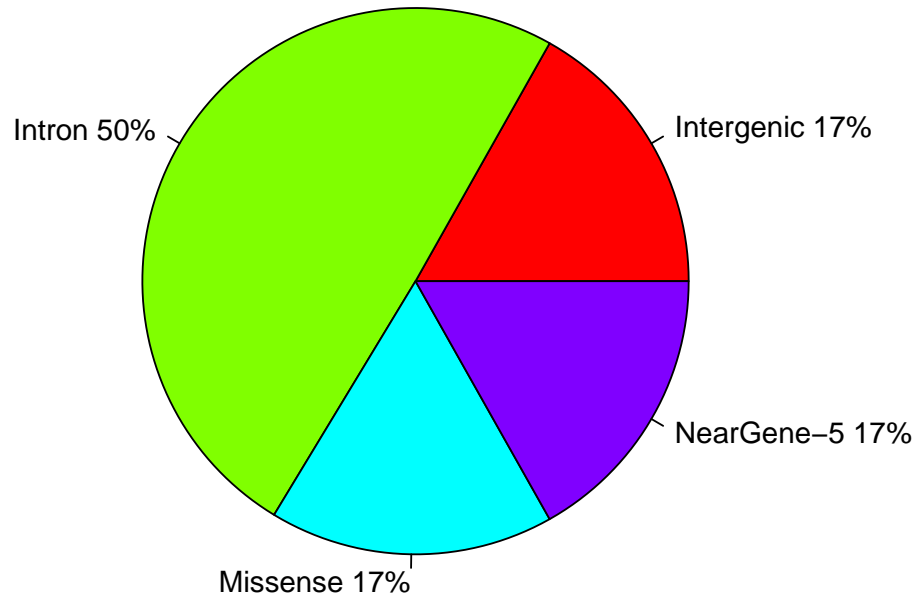
49	112
379	236
62	15

### 3.4 Exploratory and visualization functions

Plot the distribution of SNP functional class

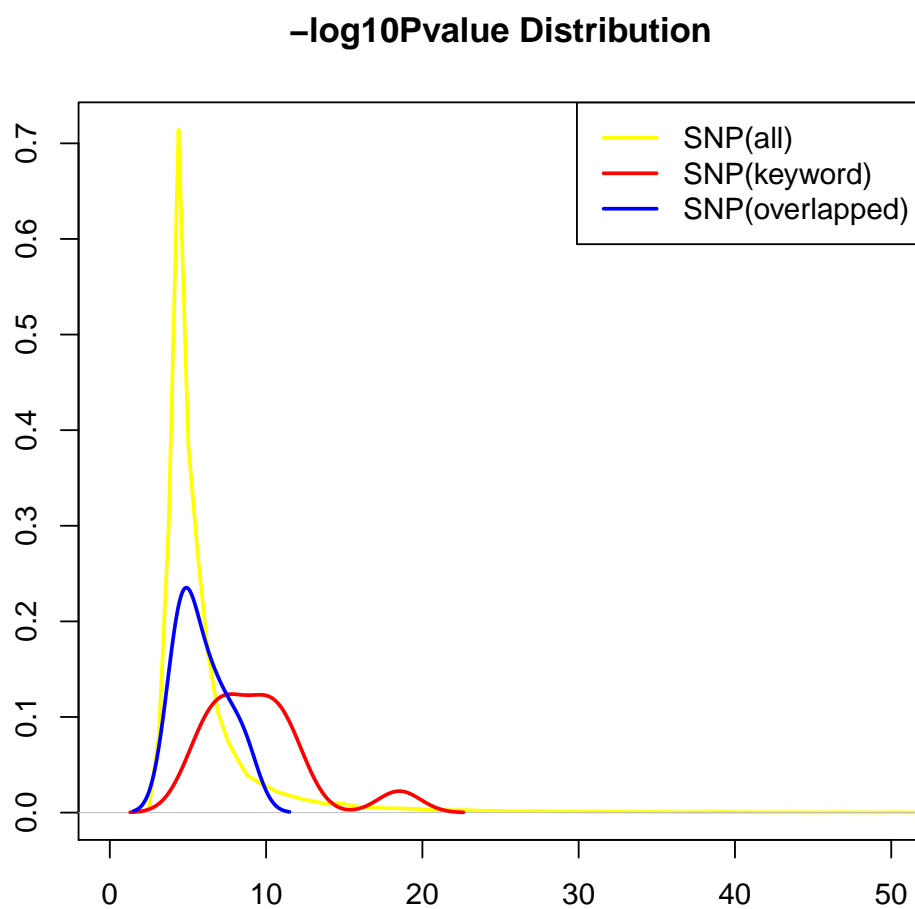
```
> plotContext(snpdb=taSNPDB,region=Tcell,keyword="Autoimmune")
```

**Pie Chart of Context**



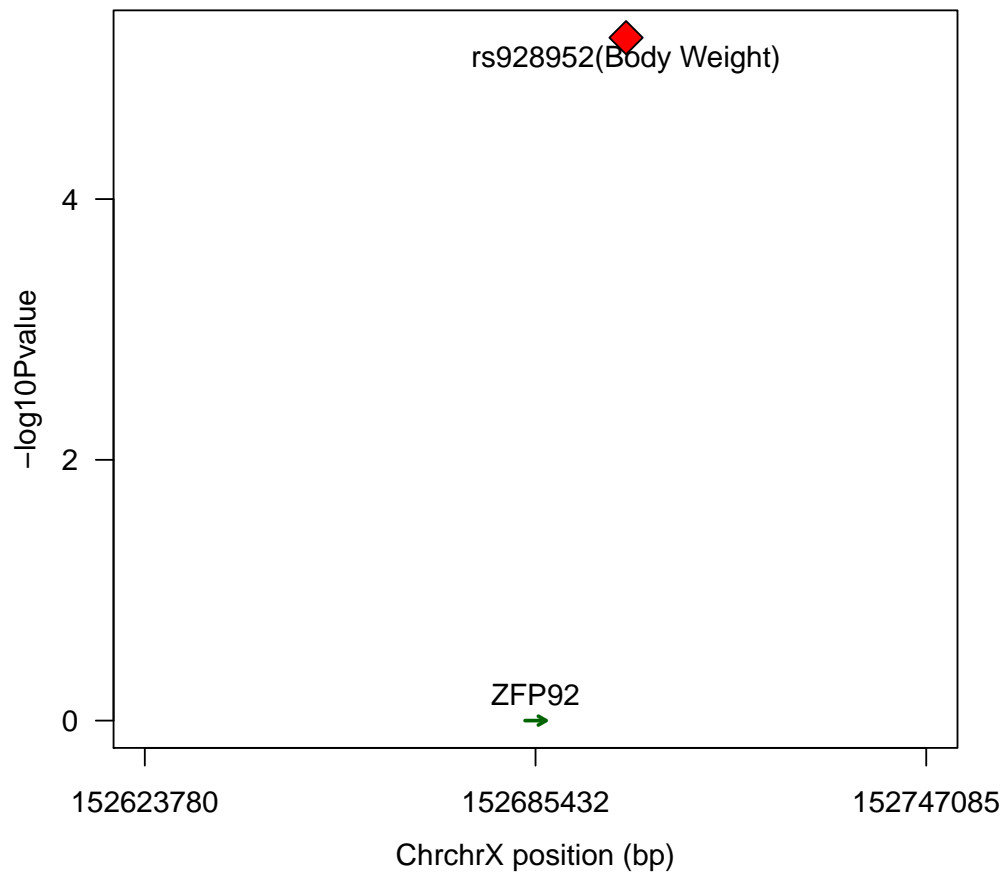
Plot the distribution of p-value of trait-associated SNPs

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



Plot SNPs or genes given genomic interval

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



Query trait-associated SNPs by key word,

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

	SNP_ID	Chr	Position	Trait.num	Trait.name
42788	rs11203203	chr21	43836186	1	Autoimmune Diseases
4923	rs1876518	chr2	65608909	1	Autoimmune Diseases
26539	rs1953126	chr9	123640500	1	Autoimmune Diseases
42919	rs2298428	chr22	21982892	1	Autoimmune Diseases
4251	rs7579944	chr2	30445026	1	Autoimmune Diseases
2186	rs864537	chr1	167411384	1	Autoimmune Diseases

Query trait-associated SNPs by gene name,

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



GRanges object with 3 ranges and 5 metadata columns:

	seqnames <Rle>	ranges <IRanges>	strand <Rle>	GENE_NAME <factor>	Trait.num <integer>	Trait.name <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 <integer>	taSNP.name <factor>
[1]	1	rs3934834
[2]	1	rs3766178
[3]	1	rs11804831

-----  
seqinfo: 23 sequences from an unspecified genome; no seqlengths

Query trait-associated SNPs by SNP name,

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

GRanges object with 2 ranges and 8 metadata columns:

	seqnames <Rle>	ranges <IRanges>	strand <Rle>	Trait <character>	SNP_ID <character>	p.value <numeric>	Context <character>
9	chr1	[1478180, 1478180]	*	Glucose	rs3766178	3.26e-05	Intron
10	chr1	[1493727, 1493727]	*	Glucose	rs880051	1.89e-05	Intron

	GENE_NAME <character>	GENE_START <integer>	GENE_END <integer>	GENE_STRAND <character>
9	SSU72	1477052	1510261	-
10	SSU72	1477052	1510261	-

-----  
seqinfo: 23 sequences from hg19 genome; no seqlengths

## 4 Conclusion

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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.

## 5 Session Info

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```
> sessionInfo()
```

R version 3.2.2 (2015-08-14)

Platform: x86\_64-apple-darwin13.4.0 (64-bit)

Running under: OS X 10.9.5 (Mavericks)

locale:

[1] C/en\_US.UTF-8/en\_US.UTF-8/C/en\_US.UTF-8/en\_US.UTF-8

attached base packages:

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

other attached packages:

[1] traseR\_1.0.0 BSgenome.Hsapiens.UCSC.hg19\_1.4.0  
[3] BSgenome\_1.38.0 rtracklayer\_1.30.0  
[5] Biostrings\_2.38.0 XVector\_0.10.0  
[7] GenomicRanges\_1.22.0 GenomeInfoDb\_1.6.0  
[9] IRanges\_2.4.0 S4Vectors\_0.8.0  
[11] BiocGenerics\_0.16.0

loaded via a namespace (and not attached):

[1] XML\_3.98-1.3 Rsamtools\_1.22.0 GenomicAlignments\_1.6.0  
[4] bitops\_1.0-6 futile.options\_1.0.0 zlibbioc\_1.16.0  
[7] futile.logger\_1.4.1 BiocStyle\_1.8.0 lambda.r\_1.1.7  
[10] BiocParallel\_1.4.0 tools\_3.2.2 Biobase\_2.30.0  
[13] RCurl\_1.95-4.7 SummarizedExperiment\_1.0.0

## References

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- [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](http://www.ncbi.nlm.nih.gov/projects/gapplusprev/sgap_plus.htm) *Association Results Browser*
- [4] <http://hapmap.ncbi.nlm.nih.gov/downloads/genotypes/latest/forward/non-redundant/> *HapMap*
- [5] <ftp://share.sph.umich.edu/1000genomes/fullProject/2012.03.14/> *1000Genome EUR*