

# SNPsea Reference Manual

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December 7, 2013

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## Introduction

SNPsea is a nonparametric permutation analysis originally conceived to test for enrichment of sample-specific expression of genes in loci near trait-associated SNPs.

The implementation described here is generalized, so you may provide a quantitative gene matrix with gene expression (or any other measurements) or a binary gene matrix with presence absence (1, 0) values. The columns of the matrix might be tissues, cell types, GO annotation codes, or any other types of conditions. In general, this analysis is appropriate when you are interested in testing for enrichment of sample-specificity of genes linked to a given set of trait-associated SNPs.

Genome-wide association analyses have identified disease and trait loci across the genome, thereby implicating many nearby linked genes.

The following hypothesis is tested by this analysis:

If trait-associated alleles impact a small number of pathogenic tissues or cell types, then the subset of genes with critical functions in those pathogenic cell types are likely to be within trait-associated loci.

We assume that a gene's specificity to a given condition is a reasonable indicator of the gene's importance to the function of that condition.

Please see the following publications for additional information outside the scope of this reference manual:

Slowikowski, K. et al. SNPsea: test trait-associated loci for enrichment of condition-specificity of gene measurements or binary annotations. Manuscript in progress.

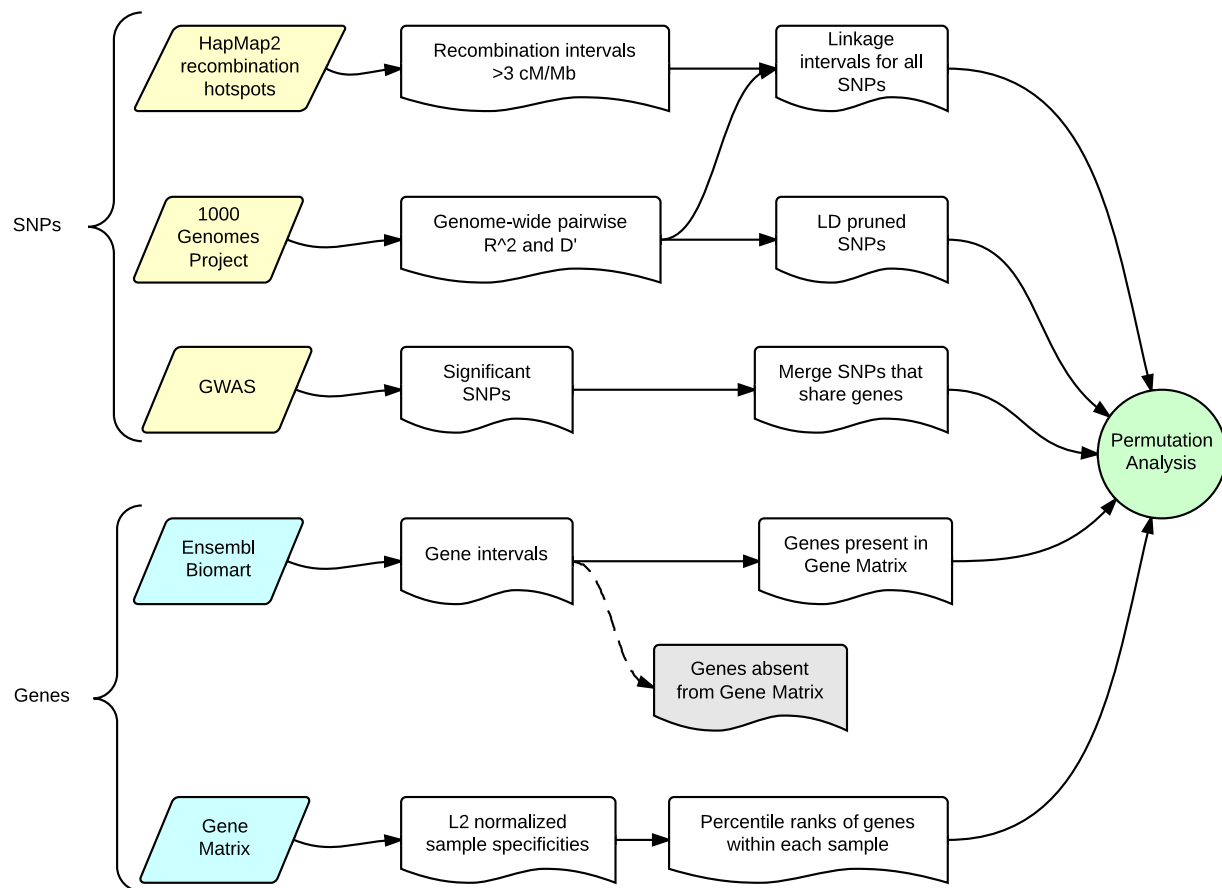
Hu, X. et al. Integrating autoimmune risk loci with gene-expression data identifies specific pathogenic immune cell subsets. The American Journal of Human Genetics 89, 496–506 (2011). [PubMed](#)

## Contact

Please contact me with questions and comments: [slowikow@broadinstitute.org](mailto:slowikow@broadinstitute.org)

## Visual Summary

### Flow Chart



This flow chart shows the input data required to perform the analysis, and a summary of the intermediate steps.

## Installation

First, install the dependencies listed below. Then run:

```
cd snpsea/src
make
```

You may move the generated executable file wherever you like:

```
mv snpsea/bin/snpsea ~/bin/
```

## Data

Download the compressed archive with data required to perform this analysis here:

[http://www.broadinstitute.org/mpg/snpsea/SNPsea\\_data\\_20131204.zip](http://www.broadinstitute.org/mpg/snpsea/SNPsea_data_20131204.zip)

Contents:

G02013.gct.gz  
Lango2010.txt.gz  
NCBIGenes2013.bed.gz  
NovartisGeneAtlas2004.gct.gz  
ImmGen2012.gct.gz  
LDL\_Teslovich2010.txt  
TGP2011.bed.gz

### **G02013.gct.gz**

A GCT formatted gene matrix with 1s and 0s indicating presence or absence of genes in Gene Ontology annotations.

### **Lango2010.txt.gz**

A list of SNPs pruned by linkage disequilibrium that span the whole genome. Null SNP sets matched on the number of genes in the user's SNP set are sampled from this list. See this paper for more information:

Lango allen H, Estrada K, Lettre G, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature. 2010;467(7317):832-8. [PubMed](#)

### **NCBIGenes2013.bed.gz**

Gene coordinates were obtained from the [NCBI](#). The coordinates I provide are a subset of those listed in `gene2refseq.gz`.

### **NovartisGeneAtlas2004.gct.gz**

Gene expression data for 79 human tissues from [GSE1133](#). Replicates for each tissue profile were averaged. For each gene, the single probe with the largest minimum was selected.

Su AI et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A, 2004 Apr 9;101(16):6062-7

**ImmGen2012.gct.gz**

Gene expression data for 249 blood cell types from [GSE15907](#). Replicates for each cell type profile were averaged. For each gene, the single probe with the largest minimum was selected.

Immunological Genome Project.

**LDL\_Teslovich2010.txt**

37 SNPs taken from:

Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010;466(7307):707-13. [PubMed](#)

**TGP2011.bed.gz**

Linkage intervals for a filtered set of SNPs from the [1000 Genomes Project](#) Phase 1 (May 21, 2011). SNP genotypes were obtained from the [BEAGLE](#) release v3 website and processed to create linkage intervals for each SNP. The linkage intervals were extended to the nearest [HapMap](#) recombination hotspot with >3 cM/Mb recombination rate.

## C++ Libraries

### [Eigen](#)

Eigen is a C++ template library for linear algebra: matrices, vectors, numerical solvers, and related algorithms.

**Instructions:** Download the latest version and unpack it. Ensure the SNPsea Makefile points to the folder that contains eigen.

### [OpenMPI](#)

MPI is a standardized API typically used for parallel and/or distributed computing. Open MPI is an open source, freely available implementation.

**Instructions:** Install on Ubuntu with:

```
sudo apt-get install libopenmpi-dev
```

### [GSL - GNU Scientific Library](#)

The GNU Scientific Library (GSL) is a numerical library for C and C++ programmers.

**Instructions:** Install on Ubuntu with:

```
sudo apt-get install libgsl0-dev
```

### [GCC, the GNU Compiler](#)

I use `c++0x` features in my C++ code, so you must use a compiler that supports them. I compiled successfully with versions 4.6.3 and 4.8.1.

## Python Packages

To plot visualizations of the results, you will need Python 2.7 and the packages listed below. Note: the packages available on the Ubuntu repositories may be outdated and might fail to work.

**Instructions:** Install with [pip](#):

```
pip install numpy pandas matplotlib
```

### [numpy](#)

NumPy is the fundamental package for scientific computing with Python.

### [pandas](#)

pandas is an open source, BSD-licensed library providing high-performance, easy-to-use data structures and data analysis tools for the Python programming language.

### [matplotlib](#)

matplotlib is a python 2D plotting library which produces publication quality figures in a variety of hardcopy formats and interactive environments across platforms.

## R Packages

Some visualizations use R and ggplot2 instead of Python and matplotlib.

**Instructions:** Start a session in R and run:

```
install.packages(c("data.table", "reshape2", "gap", "ggplot2"))
```

### [data.table](#)

Extension of data.frame for fast indexing, fast ordered joins, fast assignment, fast grouping and list columns.

### [reshape2](#)

Flexibly reshape data: a reboot of the reshape package.

### [gap](#)

Genetic analysis package.

### [ggplot2](#)

An implementation of the Grammar of Graphics.

## Usage

### Example

Here is a [Bash](#) script with a usage example:

```

options=(
  --snps LDL_Teslovich2010.txt
  --gene-matrix NovartisGeneAtlas2004.gct.gz
  --gene-intervals NCBIgenes2013.bed.gz
  --snp-intervals TGP2011.bed.gz
  --null-snps Lango2010.txt.gz
  --out out
  --slop 250e3
  --threads 4
  --null-snpsets 1e3
  --min-observations 50
  --max-iterations 1e6
)
snpsea ${options[*]} > log.txt

```

This will run the analysis on SNPs associated with LDL cholesterol and test for tissue-specific expression of the nearby genes across 79 human tissues in the Novartis 2011 gene expression matrix. Additionally, 1000 null random matched SNP sets will be tested and their results will also be recorded. Each tissue will be tested up to 1 million times, or testing will stop for a tissue if 50 matched SNP sets are observed to achieve a higher specificity score than the user's SNPs.

## Options

All input files may be optionally compressed with [gzip](#).

## Required

<code>--snps ARG</code>	Text file with SNP identifiers in the first column. Instead of a file name, you may use 'randomN' with an integer N for a random SNP list of length N.
<code>--gene-matrix ARG</code>	Gene matrix file in GCT format. The Name column must contain the same gene identifiers as in <code>--gene-intervals</code> .
<code>--gene-intervals ARG</code>	BED file with gene intervals. The fourth column must contain the same gene identifiers as in <code>--gene-matrix</code> .
<code>--snp-intervals ARG</code>	BED file with all known SNP intervals. The fourth column must contain the same SNP identifiers as in <code>--snps</code> and <code>--null-snps</code> .
<code>--null-snps ARG</code>	Text file with names of SNPs to sample when

generating null matched or random SNP sets.  
These SNPs must be a subset of `--snps-intervals`.

`--out ARG` Create output files in this directory. It will be created if it does not already exist.

## Optional

`--condition ARG` Text file with a list of columns in `--gene-matrix` to condition on before calculating p-values. Each column in `--gene-matrix` is projected onto each column listed in this file and its projection is subtracted.

`--slop ARG` If a SNP interval overlaps no gene intervals, extend the SNP interval this many nucleotides further and try again.  
[default: 250000]

`--threads ARG` Number of threads to use.  
[default: 1]

`--null-snpsets ARG` Test this many null matched SNP sets, so you can compare your results to a distribution of null results.  
[default: 10]

`--min-observations ARG` Stop testing a column in `--gene-matrix` after observing this many null SNP sets with specificity scores greater or equal to those obtained with the SNP set in `--snps`. Increase this value to obtain more accurate p-values.  
[default: 25]

`--max-iterations ARG` Maximum number of null SNP sets tested for each column in `--gene-matrix`. Increase this value to resolve smaller p-values.  
[default: 1000]

## Input File Formats

`--snps ARG`

You must provide one or more comma-separated text files. SNP identifiers must be listed one per line. Only the first column is used.

```
head LDL_Teslovich2010.txt
```



rs11136341	chr8	145043543
rs3757354	chr6	16127407
rs12027135	chr1	25775733
rs217386	chr7	44600695
rs1169288	chr12	121416650
rs7225700	chr17	45391804
rs2479409	chr1	55504650
rs247616	chr16	56989590
rs2954022	chr8	126482621
rs1564348	chr6	160578860

Instead of providing a file with SNPs, you may use “randomN” like this:

```
--snps random20
```

to sample 20 random SNPs from the **--snp-intervals** file.

#### **--gene-matrix FILE**

You must provide a single gene matrix that must be in [GCT](#) format.

```
zcat NovartisGeneAtlas2004.gct.gz | cut -f1-4 | head
```

```
#1.2
```

```
17581 79
```

Name	Description	Colorectal_Adenocarcinoma	Whole_Blood
1	A1BG	115.5	209.5
2	A2M	85	328.5
9	NAT1	499	1578
10	NAT2	115	114
12	SERPINA3	419.5	387.5
13	AADAC	125	252.5
14	AAMP	2023	942.5

#### **--condition FILE (Optional)**

You may provide column names present in the **--gene-matrix** file, one per line. The matrix will be conditioned on these columns before the analysis is performed to help you identify secondary signals independent of these columns. Binary (0, 1) matrices will not be conditioned.

```
head conditions.txt
```

```
Whole_Blood
```

### **--gene-intervals FILE**

You must provide gene intervals in BED format with a fourth column that contains the same gene identifiers as those present in the Name column of the **--gene-matrix** GCT file. Only the first four columns are used.

```
zcat NCBIgenes2013.bed.gz | head
```

```
chr1 10003485 10045555 64802 NMNAT1
chr1 100111430 100160096 54873 PALMD
chr1 100163795 100164756 100129320 HMGB3P10
chr1 100174205 100232185 391059 FRRS1
chr1 10027438 10027515 100847055 MIR5697
chr1 100308165 100308317 100270894 RPL39P9
chr1 100315632 100389578 178 AGL
chr1 100433941 100435837 730081 LOC730081
chr1 100435344 100492534 23443 SLC35A3
chr1 100503669 100548932 64645 HIAT1
```

### **--snp-intervals FILE**

SNP linkage intervals must be specified in BED format and include a fourth column with the SNP identifiers. The linkage intervals assigned to the trait-associated SNPs you provide with **--snps** are taken from this file.

```
zcat TGP2011.bed.gz | head
```

```
chr1 0 254996 rs113759966
chr1 0 254996 rs114420996
chr1 0 254996 rs114608975
chr1 0 254996 rs115209712
chr1 0 254996 rs116400033
chr1 0 254996 rs116504101
chr1 0 254996 rs12184306
chr1 0 254996 rs12184307
chr1 0 254996 rs138808727
chr1 0 254996 rs139113303
```

### **--null-snps FILE**

The null SNPs file must have one SNP identifier per line. Only the first column is used. The identifiers must be a subset of the identifiers in **--snp-intervals**.

```
zcat Lango2010.txt.gz | head
```

```
rs58108140 chr1 10583
```

rs180734498	chr1	13302
rs140337953	chr1	30923
rs141149254	chr1	54490
rs2462492	chr1	54676
rs10399749	chr1	55299
rs189727433	chr1	57952
rs149755937	chr1	59040
rs77573425	chr1	61989
rs116440577	chr1	63671

## Output Files

The usage example shown above produces the following output files:

```
out/
  args.txt
  pvalues.txt
  null_pvalues.txt
  snp_genes.txt
  snp_pvalues.txt
```

### args.txt

The command line arguments needed to reproduce the analysis.

```
cat args.txt

snpssea --snps LDL_Teslovich2010.txt
        --gene-matrix NovartisGeneAtlas2004.gct.gz
        --gene-intervals NCBIgenes2013.bed.gz
        --snp-intervals TGP2011.bed.gz
        --null-snps Lango2010.txt.gz
        --out out
        --slop 250000
        --threads 4
        --null-snpsets 1000
        --min-observations 50
        --max-iterations 1000000
```

### pvalues.txt

The p-values representing enrichment of sample-specificity for the given SNPs.

```
head pvalues.txt | column -t
```

name	pvalue	nulls_observed	nulls_tested
ColorectalAdenocarcinoma	0.87	87	100
WholeBlood	0.00606299	77	12700
BM-CD33+Myeloid	0.054	81	1500
PB-CD14+Monocytes	0.316667	95	300
PB-BDCA4+Dentritic_Cells	0.164286	115	700
PB-CD56+NKCells	0.000104993	86	819100

#### null\_pvalues.txt

If the argument for **--snps** is the name of a file, the p-values for null matched SNP sets. You can compare these null results to the results for your trait-associated SNPs.

If the argument for **--snps** is “randomN” where N is some integer, like “random20” the p-values for random unmatched SNP sets, each with N SNPs.

The fifth column is the replicate index. The number of replicates performed is specified with **--null-snpsets INT**.

```
head null_pvalues.txt | column -t
```

ColorectalAdenocarcinoma	0.056	84	1500	0
WholeBlood	0.236667	71	300	0
BM-CD33+Myeloid	0.55	55	100	0
PB-CD14+Monocytes	0.59	59	100	0
PB-BDCA4+Dentritic_Cells	0.59	59	100	0
PB-CD56+NKCells	0.71	71	100	0
PB-CD4+Tcells	0.383333	115	300	0
PB-CD8+Tcells	0.128571	90	700	0
PB-CD19+Bcells	0.168571	118	700	0
BM-CD105+Endothelial	0.386667	116	300	0

#### snp\_genes.txt

Each SNP’s linkage interval and overlapping genes. If a SNP is not found in the reference file specified with **--snp-intervals**, then the name of the SNP will be listed and the other columns will contain NA.

```
head snp_genes.txt | column -t
```

chrom	start	end	name	n_genes	genes
chr7	128560761	128773770	rs10488631	2	3663,23534
chr2	100637056	100895271	rs10865035	3	3899,150577,164832
chr11	118475098	118746223	rs10892279	2	1656,23187
NA	NA	NA	rs99999999	NA	NA
chr21	43817297	43851877	rs11203203	1	53347
chr1	117256697	117293763	rs11586238	4	914,965,3321,5738

chr1	161389417	161637888	rs12746613	5	2212,2213,2215,3310,9103
chr2	61068167	61382443	rs13031237	5	5194,5966,84542,339803,339804
chr3	58553160	58558769	rs13315591	1	11170

#### **snp\_pvalues.txt**

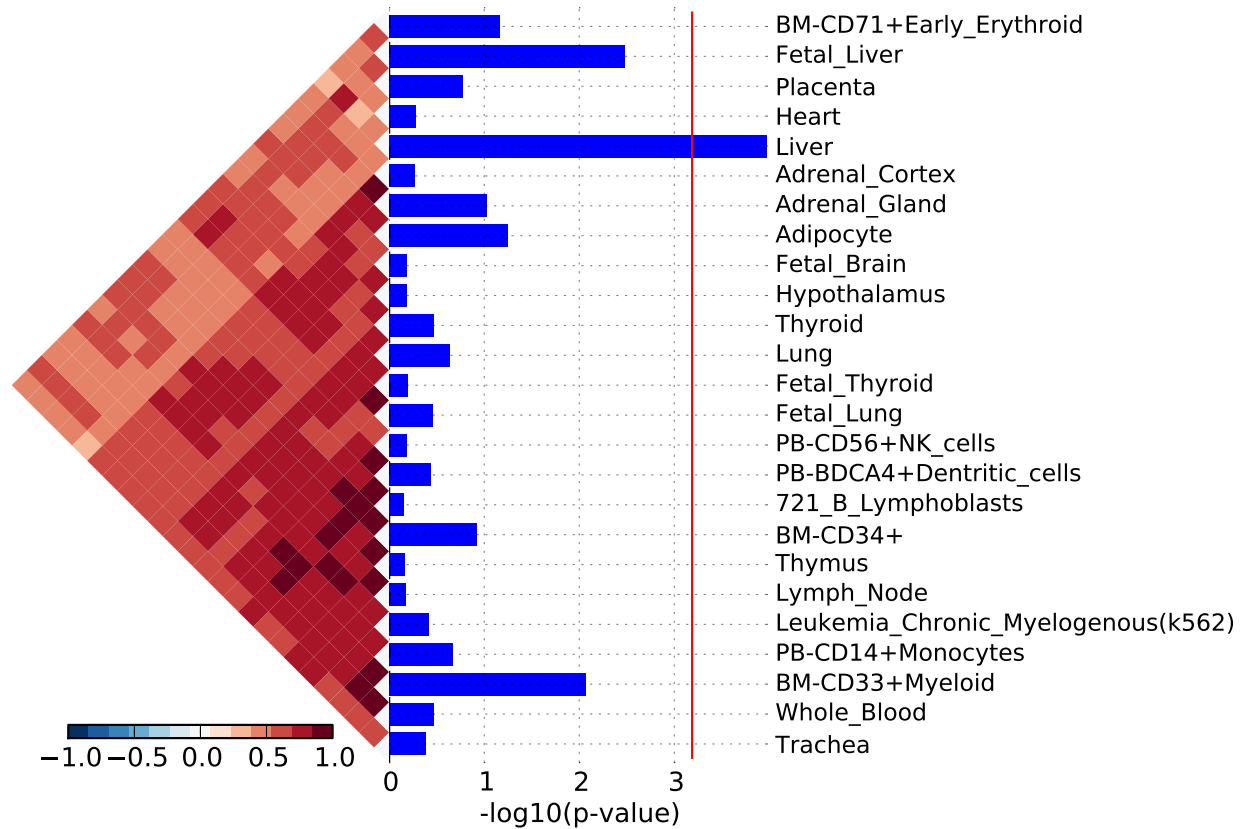
Each SNP, sample, gene with greatest specificity to that sample, and p-value for the SNP-sample pair, adjusted for the number of genes overlapping the given SNP.

```
head snp_pvalues.txt | column -t
```

marker	column	gene	pvalue
rs10488631	ColorectalAdenocarcinoma	3663	0.270409
rs10488631	WholeBlood	3663	0.302693
rs10488631	BM-CD33+Myeloid	3663	0.0569547
rs10488631	PB-CD14+Monocytes	3663	0.0960891
rs10488631	PB-BDCA4+Dentritic_Cells	3663	0.240571
rs10488631	PB-CD56+NKCells	23534	0.58674
rs10488631	PB-CD4+Tcells	3663	0.683486
rs10488631	PB-CD8+Tcells	23534	0.634216
rs10488631	PB-CD19+Bcells	3663	0.261931

## Output Visualizations

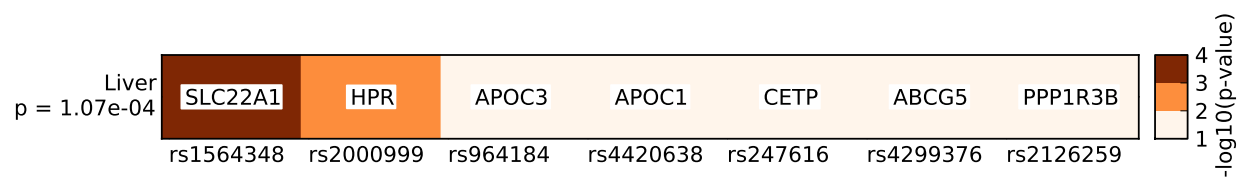
View enrichment of tissue-specific gene expression



Create this visualization with:

```
python bin/barplot.py --out out
```

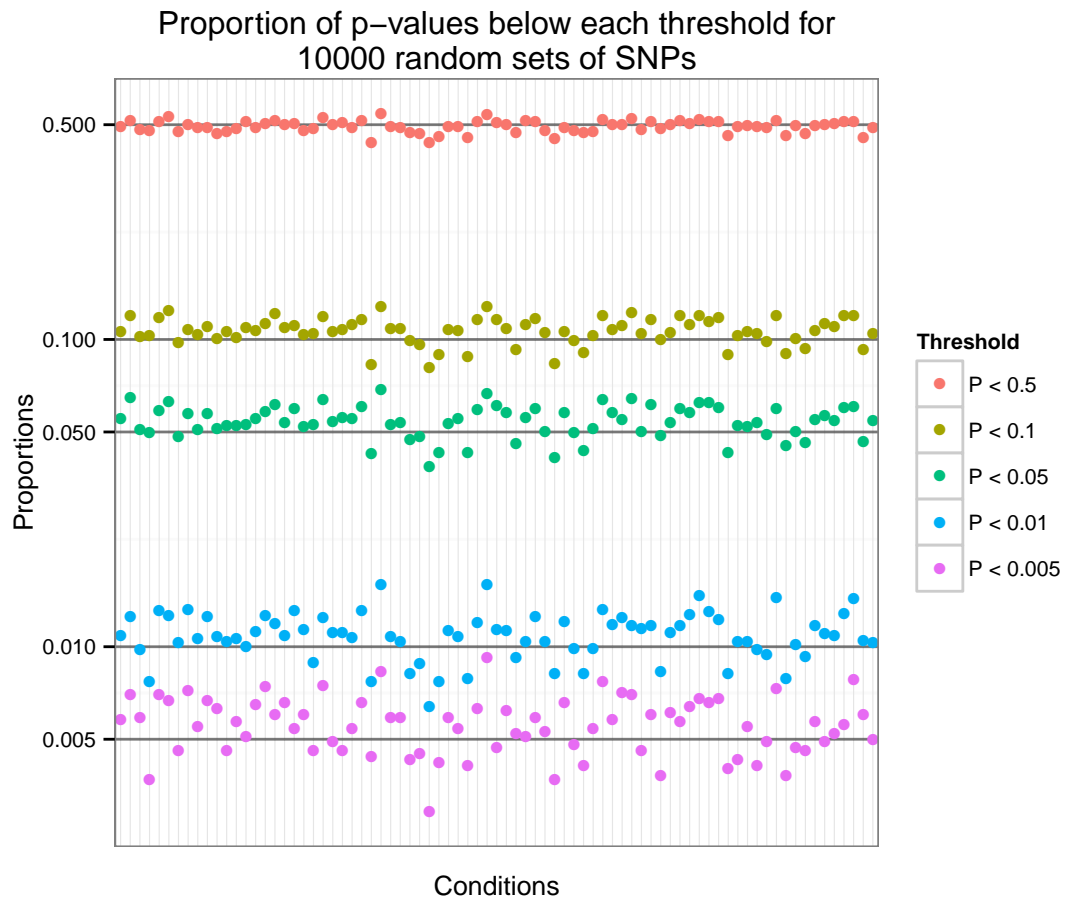
View the most specifically expressed gene for each SNP-tissue pair



Create this visualization with:

```
python bin/heatmap.py --out out
```

View the type 1 error rate estimates for each tissue



Create this visualization with:

```
Rscript bin/type1error.R out
```