SNPsea Reference Manual

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Introduction

SNPsea is a nonparametric permutation analysis for identifying pathways and tissue types influenced by the alleles discovered through genome-wide association studies (GWAS). It was originally conceived to test for enrichment of cell type-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 condition-specificity of genes linked to a given set of trait-associated SNPs.

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 cell type or condition is a reasonable indicator of the gene's importance to the function of that cell type.

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.

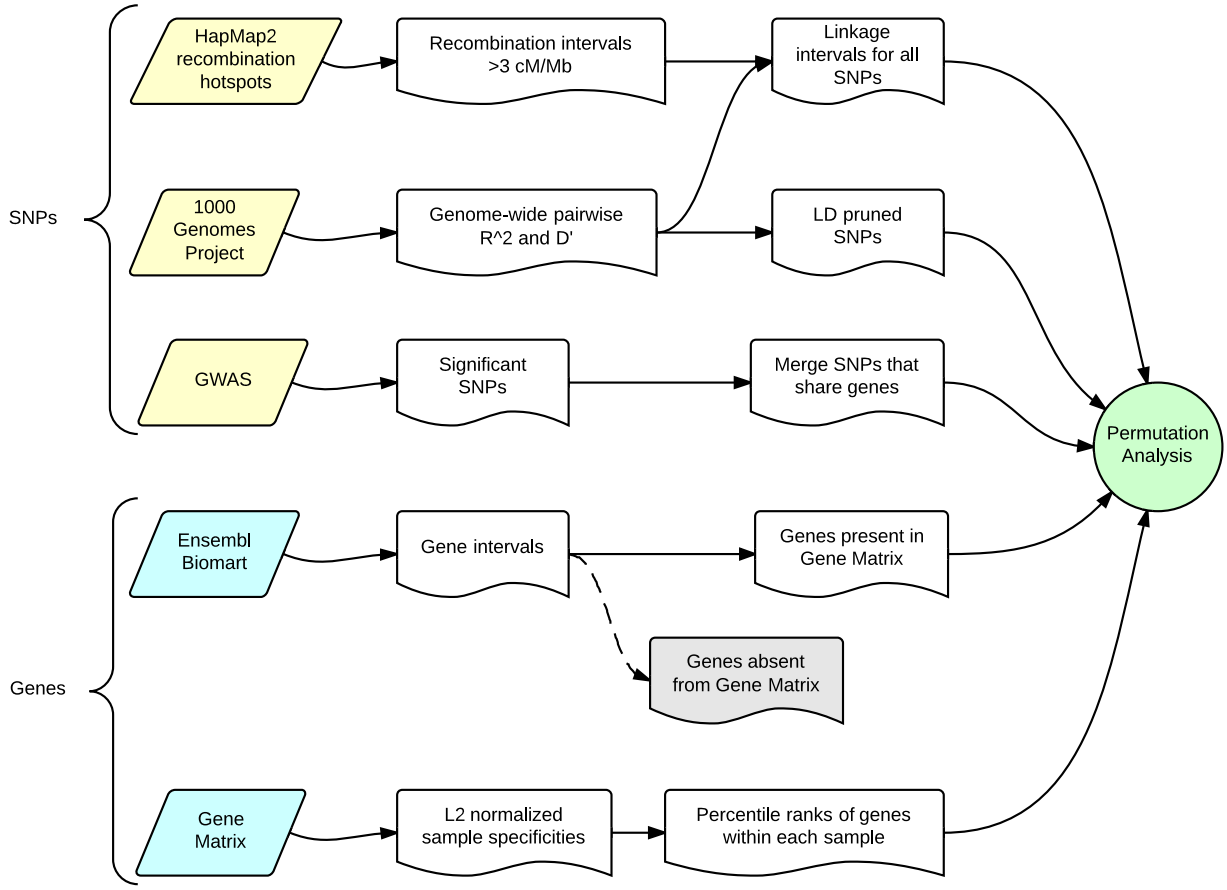
> Hu, X. et al. Integrating autoimmune risk loci with gene-expression data identifies specific pathogenic immune cell subsets. The American Journal of Human Genetics 89, 496–506 (2011). PubMed

Contact

Please contact me with questions and comments: slowikow@broadinstitute.org

Visual Summary

Flow Chart



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

Installation

Download the binary: https://github.com/slowkow/snpsea/releases

Or, you can download the source code: https://github.com/slowkow/snpsea

After you install the dependencies listed below, 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 (138M):

http://dx.doi.org/10.6084/m9.figshare.871430

Contents:

```
GO2013.gct.gz
ImmGen2012.gct.gz
LDL_Teslovich2010.txt
Lango2010.txt.gz
NCBIgenes2013.bed.gz
NovartisGeneAtlas2004.gct.gz
TGP2011.bed.gz
```

`GO2013.gct.gz`

A GCT formatted gene matrix with 1s and 0s indicating presence or absence of 19,111 genes in 1,751 Gene Ontology annotations.

`ImmGen2012.gct.gz`

Gene expression data for 15,139 genes across 249 blood cell types from GSE15907. Replicates for each cell type were averaged. For each gene, the single probe with the largest minimum was selected.

> Immunological Genome Project. http://www.immgen.org/

`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

`Lango2010.txt.gz`

A list of 56,890 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`

40,437 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 17,581 genes across 79 human tissues from GSE1133. Replicates for each tissue 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

`TGP2011.bed.gz`

Linkage intervals for a filtered set of 22,518,294 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 docopt numpy pandas matplotlib
```

docopt

Command-line interface description language.

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.

Note: On a server with no display, please edit your matplotlibrc file to use the `Agg` backend. Otherwise, you may see an error message like this:

```
_tkinter.TclError: no display name and no $DISPLAY environment variable
```

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

```
--snps ARG                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.

--gene-matrix ARG         Gene matrix file in GCT format. The Name column
                          must contain the same gene identifiers as in
                          --gene-intervals.

--gene-intervals ARG      BED file with gene intervals. The fourth column
                          must contain the same gene identifiers as in
                          --gene-matrix.
```

--snp-intervals ARG
: BED file with all known SNP intervals. The fourth column must contain the same SNP identifiers as in --snps and --null-snps.

--null-snps ARG
: Text file with names of SNPs to sample when generating null matched or random SNP sets. These SNPs must be a subset of --snp-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 Langо2010.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
```

```
snpsea --snps LDL_Teslovich2010.txt
       --gene-matrix NovartisGeneAtlas2004.gct.gz
       --gene-intervals NCBIgenes2013.bed.gz
       --snp-intervals TGP2011.bed.gz
       --null-snps Langо2010.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 condition-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

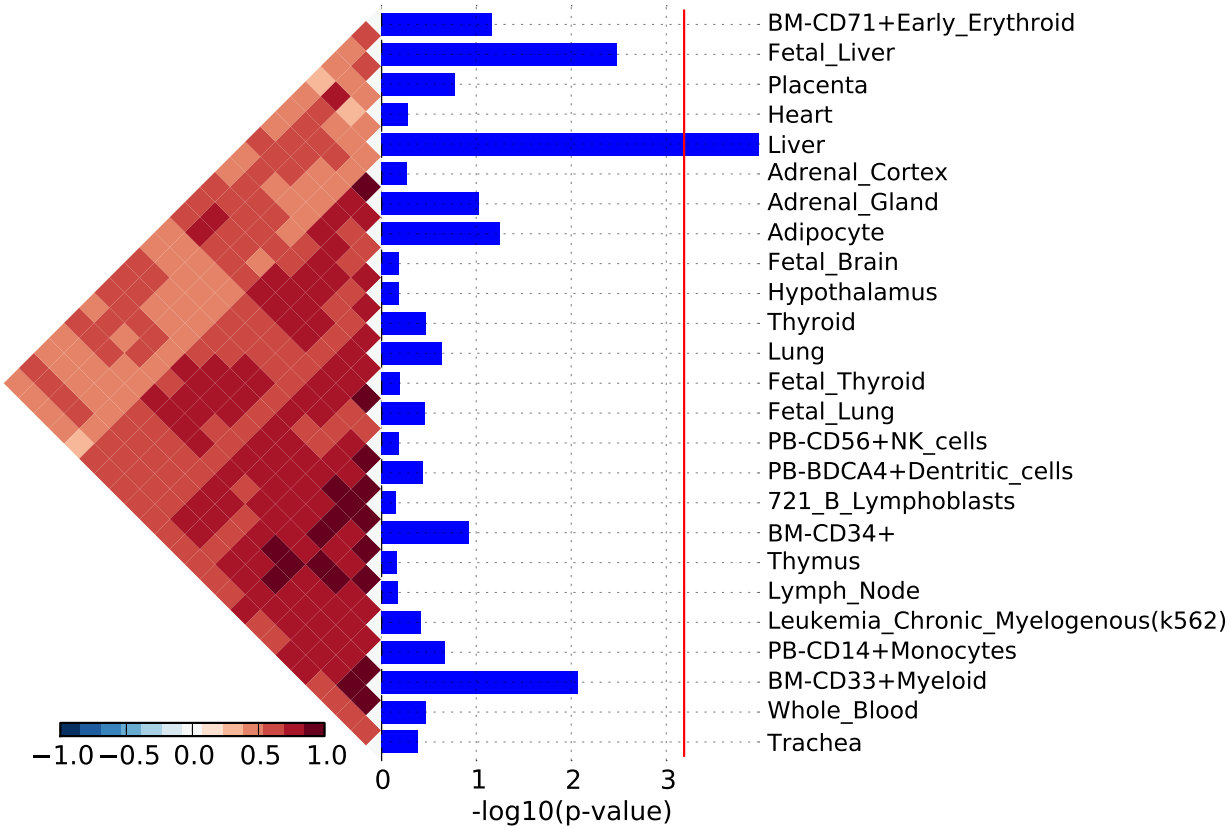
Each SNP, condition, gene with greatest specificity to that sample, and p-value for the SNP-sample pair, adjusted for the number of genes overlapping the given SNP.

```
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

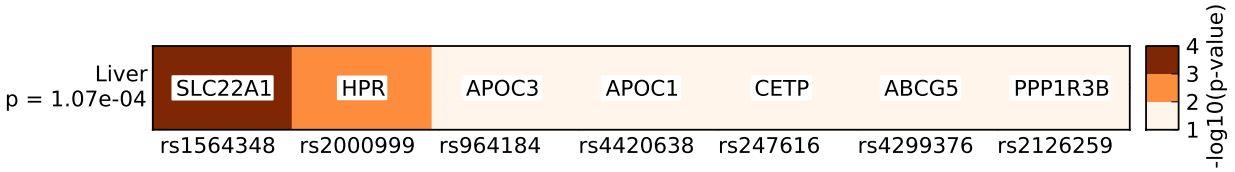
View enrichment of tissue-specific gene expression



Create this visualization with:

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

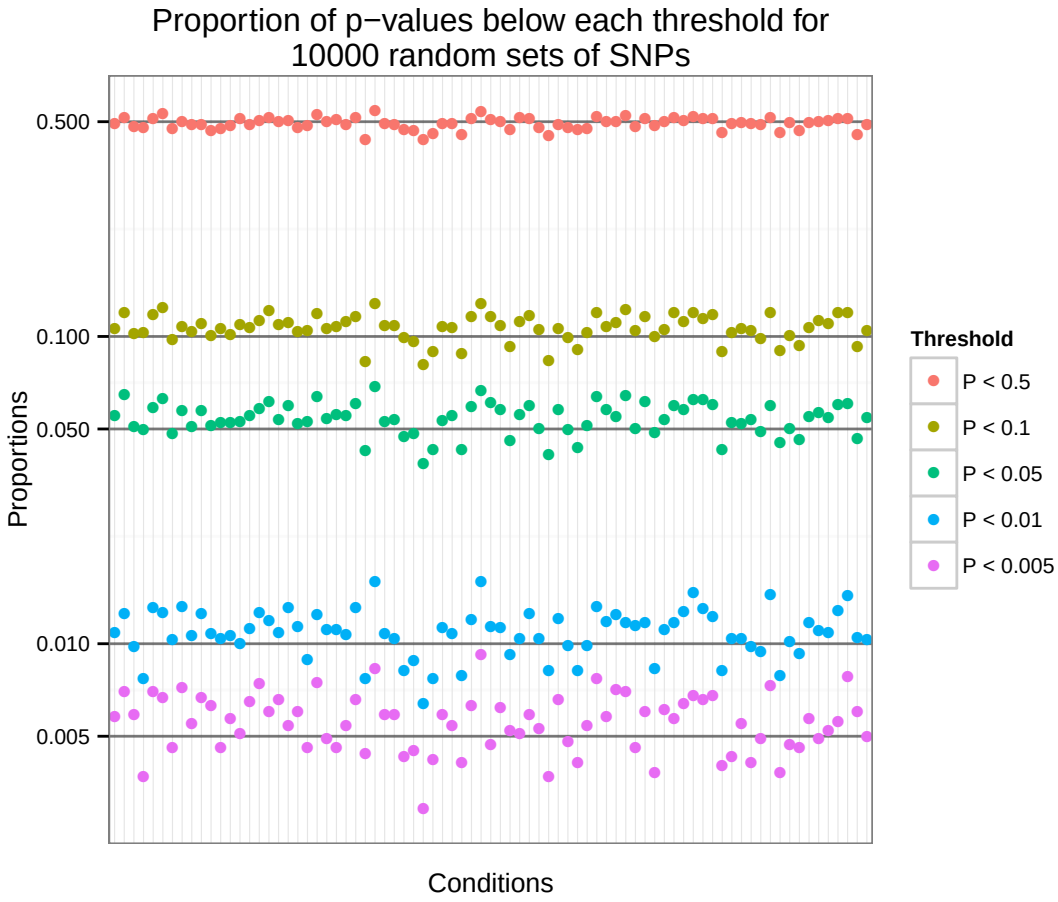
View the most specifically expressed gene for each SNP-tissue pair



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
```