SNPsea Reference Manual

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Contents

Introduction

SNPsea is a general algorithm to identify cell types, tissues, and pathways likely to be affected by risk loci.

For example, with a gene expression matrix containing expression profiles for multiple cell types, we identify genes in linkage disequilibrium with trait-associated SNPs and score them for specificity to each cell type. We compare the score to a null distribution by sampling random SNP sets matched on the number of linked genes. To evaluate significance, we calculate an exact permutation p-value.

This implementation is generalized, so you may provide (1) a continuous gene matrix with gene expression (or any other values) or (2) a binary gene matrix with presence/absence 1/0 values.

The columns of the matrix could be tissues, cell types, GO annotation codes, or any other types of *conditions*. Continuous matrices *must* be normalized before running SNPsea so that columns are directly comparable to each other.

In general, this analysis is appropriate when you are interested in testing for enrichment of condition-specificity of genes linked to a set of trait-associated SNPs.

If trait-associated alleles impact a small number of pathogenic tissues or cell types, we hypothesize that 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 its function.

If you benefit from this method, please cite:

> Slowikowski, K. et al. SNPsea: an algorithm to identify cell types, tissues, and pathways affected by risk loci > Manuscript in progress.

See additional examples:

> 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

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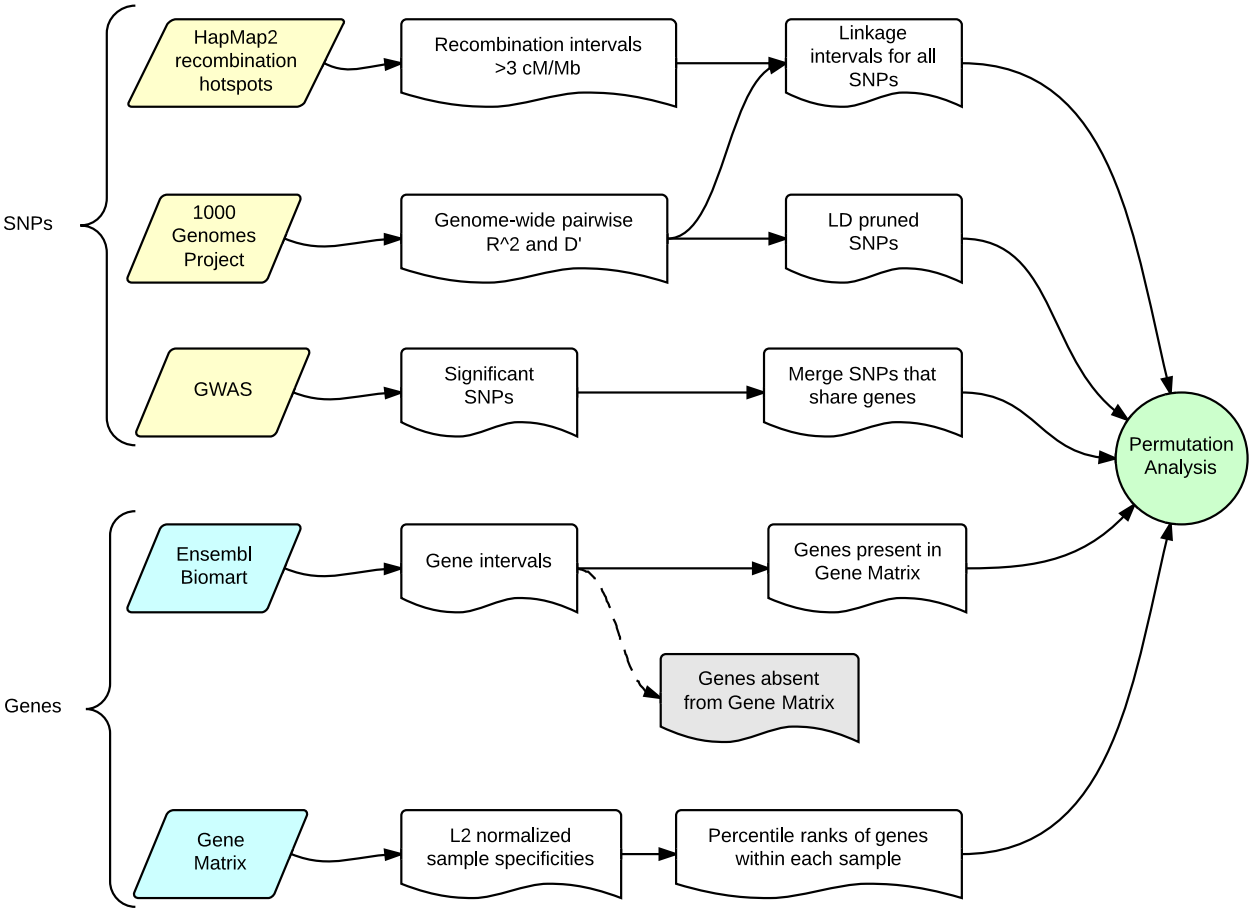
Visual Summary

Flow Chart



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

Installation

Linux 64-bit

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

Mac

To compile C++ code with the required dependencies, you need XCode and MacPorts: http://guide.macports.org/#installing.xcode

All other platforms

The source code is available: https://github.com/slowkow/snpsea

Install the dependencies:

```
# Ubuntu

sudo apt-get install build-essential libopenmpi-dev libgsl0-dev

# Mac

#   Install MacPorts: http://www.macports.org/
#   Then do this:
sudo port selfupdate
sudo port install gcc48 openmpi gsl

# Broad Institute

#   You can add this to your .my.bashrc or .my.cshrc
use .gcc-4.8.1 .openmpi-1.4 .gsl-1.14
```

Download and compile the code:

```
#   Clone with git, so you can get updates with 'git pull'
git clone https://github.com/slowkow/snpsea.git
cd snpsea

#   or if you don't have git
curl -LOk https://github.com/slowkow/snpsea/archive/master.zip
unzip master.zip
cd snpsea-master

#   Compile.
cd src
make

#   Move the executables wherever you like.
mv ../bin/snpsea* ~/bin/
```

Download the required data and run SNPsea:

```
mkdir ../snpsea/data
cd ../snpsea/data
curl -LOk http://files.figshare.com/1307287/SNPsea_data_20131204.zip
unzip SNPsea_data_20131204.zip

snpsea
```

Data

Download the compressed archive with data required to perform this analysis here (138M):

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

```
GWAS SNPs

Celiac_disease-Trynka2011-35_SNPs.gwas
HDL_cholesterol-Teslovich2010-46_SNPs.gwas
Multiple_sclerosis-IMSGC-51_SNPs.gwas
Red_blood_cell_count-Harst2012-45_SNPs.gwas


GeneAtlas2004.gct.gz  # Gene Atlas 2004 gene expression matrix
GO2013.gct.gz         # Gene Ontology 2013 gene annotation matrix
ImmGen2012.gct.gz     # ImmGen 2012 gene expression matrix


NCBIgenes2013.bed.gz  # NCBI gene intervals
Lango2010.txt.gz      # LD-pruned SNPs
TGP2011.bed.gz        # 1000 Genomes Project SNP linkage intervals
```

Celiac_disease-Trynka2011-35_SNPs.gwas

35 SNPs associated with Celiac disease taken from Table 2. Positions are on hg19. All SNPs have P <= 5e-8.

```
doi:10.1038/ng.998
PMID: 22057235

Trynka G, Hunt KA, Bockett NA, et al. Dense genotyping identifies and
localizes multiple common and rare variant association signals in celiac
disease. Nat Genet. 2011;43(12):1193-201.

http://www.ncbi.nlm.nih.gov/pubmed/22057235
```

HDL_cholesterol-Tesolvich2010-46_SNPs.gwas

46 SNPs associated with HDL taken from Supplementary Table 2. Positions are on hg19. All SNPs have P <= 5e-8.

```
doi:10.1038/nature09270
PMID: 20686565

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.

http://www.ncbi.nlm.nih.gov/pubmed/20686565
```

Multiple_sclerosis-IMSGC-51_SNPs.gwas

51 SNPs associated with Multiple Sclerosis taken from Supplementary Table A. Positions are on hg19. All SNPs have P <= 5e-8.

doi:10.1038/nature10251
PMID: 21833088

Sawcer S, Hellenthal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011;476(7359):214-9.

http://www.ncbi.nlm.nih.gov/pubmed/21833088

Red_blood_cell_count-Harst2012-45_SNPs.gwas

45 SNPs associated with red blood cell count (RBC) taken from Table 1. Positions are on hg19. All SNPs have P <= 5e-8.

doi:10.1038/nature11677
PMID: 23222517

Van der harst P, Zhang W, Mateo leach I, et al. Seventy-five genetic loci influencing the human red blood cell. Nature. 2012;492(7429):369-75.

http://www.ncbi.nlm.nih.gov/pubmed/23222517

GeneAtlas2004.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

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1133

GO2013.gct.gz

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

http://www.geneontology.org/

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

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE15907
```

NCBIgenes2013.bed.gz

All human start and stop positions taken from:

```
ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/gene2refseq.gz
```

Lango2010.txt.gz

A list of SNPs that span the whole genome, pruned by linkage disequilibrium (LD). SNPsea samples null SNP sets matched on the number of genes in the user's SNP set 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.

http://www.ncbi.nlm.nih.gov/pubmed/20881960
```

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.

```
http://www.1000genomes.org/
http://bochet.gcc.biostat.washington.edu/beagle/1000_Genomes.phase1_release_v3/
http://hapmap.ncbi.nlm.nih.gov/downloads/
```

C++ Libraries

To compile SNPsea, you will need a modern C++ compiler that supports `c++0x` and the dependencies listed below.

See: Installation

intervaltree

a minimal C++ interval tree implementation

Eigen

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

OpenMPI

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

GSL - GNU Scientific Library

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

GCC, the GNU Compiler

The GNU Compiler Collection is a compiler system produced by the GNU Project supporting various programming languages.

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 (the default version for Ubuntu 12.04) and 4.8.1.

Python Packages

To plot visualizations of the results, you will need Python 2.7 and the packages listed below.

Instructions: Install with pip:

```
pip install docopt numpy pandas matplotlib
```

Note: The packages available on the Ubuntu repositories may be outdated and might fail to work. So, avoid using `apt-get` for these dependencies.

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:

```
perl -i -pe 's/^(\s*(backend).*)$/#$1\n$2:Agg/' ~/.matplotlib/matplotlibrc
```

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       GeneAtlas2004.gct.gz
    --gene-intervals    NCBIgenes2013.bed.gz
    --snp-intervals     TGP2011.bed.gz
    --null-snps         Lango2010.txt.gz
    --out               out
    --slop              10e3
    --threads           4
    --null-snpsets      0
    --min-observations  50
    --max-iterations    1e6
)
snpsea ${options[*]}
```

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 Gene Atlas 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 optionally be 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: 10000]

--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: 0]

--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: 10000]

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 ARG`

You must provide a single gene matrix that must be in GCT format.

```
zcat GeneAtlas2004.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 ARG (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 ARG

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 ARG

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 ARG

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
    condition_pvalues.txt
    null_pvalues.txt
    snp_condition_scores.txt
    snp_genes.txt
```

args.txt

The command line arguments needed to reproduce the analysis.

```
cat args.txt
```

```
# SNPsea v1.0.2
--snps              Red_blood_cell_count-Harst2012-45_SNPs.gwas
--gene-matrix       GeneAtlas2004.gct.gz
--gene-intervals    NCBIgenes2013.bed.gz
--snp-intervals     TGP2011.bed.gz
--null-snps         Lango2010.txt.gz
--out               out
--score             single
--slop              100000
--threads           8
--null-snpsets      0
```

```
--min-observations 100
--max-iterations   10000000
```

Repeat the analysis:

```
snpsea --args args.txt
```

condition_pvalues.txt

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

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

condition	pvalue	nulls_observed	nulls_tested
Colorectal_Adenocarcinoma	0.933555	280	300
Whole_Blood	0.521595	156	300
BM-CD33+Myeloid	0.159772	111	700
PB-CD14+Monocytes	0.103264	154	1500
PB-BDCA4+Dentritic_cells	0.0606256	187	3100
PB-CD56+NK_cells	0.194009	135	700
PB-CD4+T_cells	0.428571	128	300
PB-CD8+T_cells	0.531561	159	300
PB-CD19+B_cells	0.226819	158	700

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
chr4   55364224   55408999   rs218238    0        NA
chr6   139827777  139844854  rs590856    0        NA
NA     NA         NA         rs99999999  NA       NA
chr6   109505894  109651220  rs1008084   2        8763,27244
chr10  71089843   71131638   rs10159477  1        3098
chr2   111807303  111856057  rs10207392  1        55289
chr16  88831494   88903796   rs10445033  4        353,2588,9780,81620
chr7   151396253  151417368  rs10480300  1        51422
chr12  4320955    4336783    rs10849023  2        894,57103
chr15  76129642   76397903   rs11072566  4        26263,92912,123591,145957
```

snp_condition_scores.txt

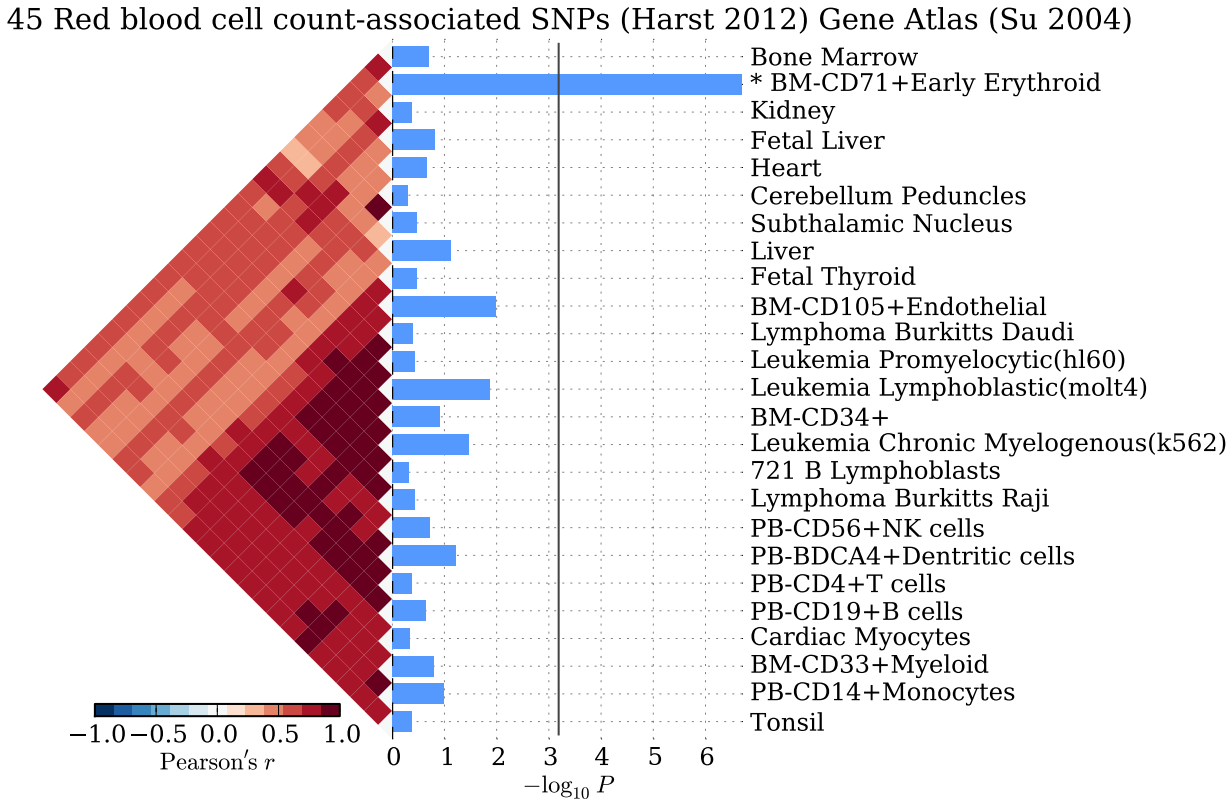
Each SNP, condition, gene with greatest specificity to that condition, and score for the SNP-condition pair, adjusted for the number of genes overlapping the given SNP's linkage interval.

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

```
snp        condition                 gene   score
rs9349204  Colorectal_Adenocarcinoma  10817  0.693027
rs9349204  Whole_Blood                896    0.285864
rs9349204  BM-CD33+Myeloid            896    0.236487
rs9349204  PB-CD14+Monocytes          29964  0.340561
rs9349204  PB-BDCA4+Dentritic_cells   29964  0.411727
rs9349204  PB-CD56+NK_cells           896    0.0356897
rs9349204  PB-CD4+T_cells             896    0.38182
rs9349204  PB-CD8+T_cells             896    0.332008
rs9349204  PB-CD19+B_cells            29964  0.255196
```

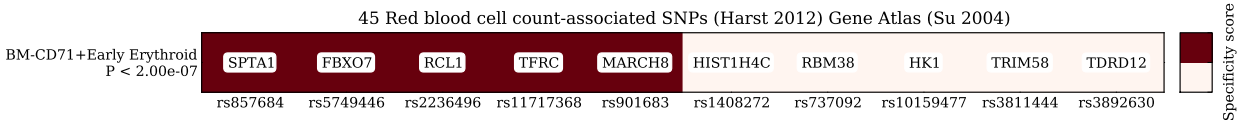
Output Visualizations

View enrichment of tissue-specific gene expression



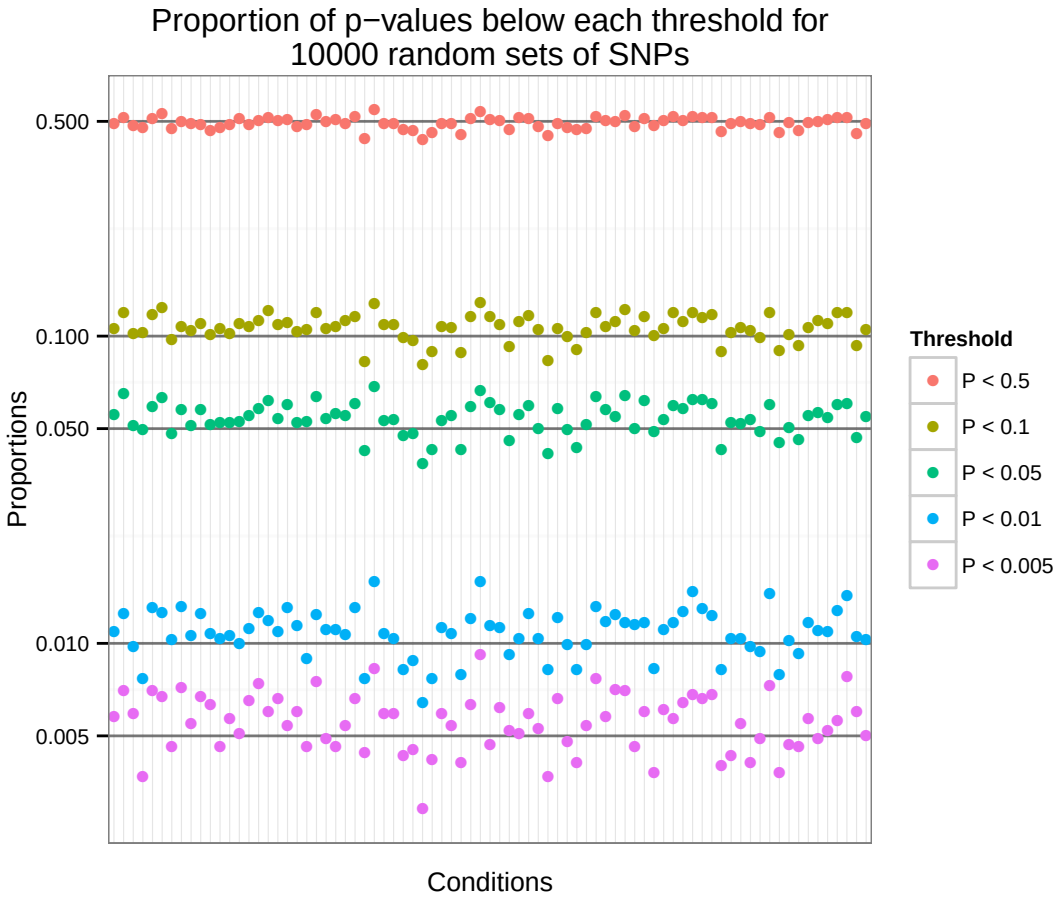

```
python bin/snpsea-barplot out
```

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




```
python bin/snpsea-heatmap out
```

View the type 1 error rate estimates for each tissue




```
Rscript bin/snpsea-type1error out
```