Overview

About

This software tool implements the SMR & HEIDI methods to test for pleiotropic association between the expression level of a gene and a complex trait of interest using summary-level data from GWAS and expression quantitative trait loci (eQTL) studies (Zhu et al. 2016 Nat Genet). The methodology can be interpreted as an analysis to test if the effect size of a SNP on the phenotype is mediated by gene expression. This tool can therefore be used to prioritize genes underlying GWAS hits for follow-up functional studies.

Credits

Futao Zhang developed the software tool and webpages with supports from Zhili Zheng, Zhihong Zhu, Ting Qi and Jian Yang.

Zhihong Zhu and Jian Yang developed the SMR and HEIDI methods.

Ting Qi and Jian Yang developed the MeCSmethod.

Questions and Help Requests

Bug reports or questions to Jian Yang (jian.yang@uq.edu.au) at Institute for Molecular Bioscience, The University of Queensland.

Citations

Zhihong Zhu, Futao Zhang, Han Hu, Andrew Bakshi, Matthew R. Robinson, Joseph E. Powell, Grant W. Montgomery, Michael E. Goddard, Naomi R. Wray, Peter M. Visscher and Jian Yang (2016) Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat Genet*, 48:481-487.

Download

Executable Files (version 0.69)

smr_Linux.zip

smr_Mac.zip

smr_Win.zip

The executable files (binary code) are released under MIT lincense.

eQTL summary data

 # Westra eQTL summary data

Westra eQTL summary data ([Westra et al. 2013 Nat Genet]) in SMR binary (BESD) format:

westra_eqtl_data_hg18.zip (hg18)

westra_eqtl_data_hg19.zip (hg19)

 # CAGE eQTL summary data

CAGE eQTL summary data ([Luke R. Lloyd-Jones et al. 2017 AJHG]) in SMR binary (BESD) format:

cage_eqtl_data_hg19.tgz (hg19)

The CAGE eQTL results have finer coverage than the Westra et al. 2013 results with comparable power. Please note that the EGCUT cohort is common to both the Westra et al. 2013 and CAGE data sets. Please see the above link to the CAGE paper that outlines how these eQTL results were generated.

Please see the associated Shiny App for further interactive interrogation of the results generated in the CAGE analysis.

R script for SMR locus plot

R script and sample file for SMR locus plot:

plot.zip

Update log

1. 24 Aug, 2015: first release.

2. 17 Sept, 2015: updated the format of sparse besd file; added a function to make sparse besd file by extracting information from full dense besd file; added a function to check quickly how many probes are associated with a SNP at p < a threshold(e.g. 5e-8).

3. 12 Oct, 2015: Eigen library and OpenMP were used.

4. Version 0.6 (10 Nov, 2015): added features of SMR and HEIDI test for the trans regions.

5. Version 0.619 (4 Aril, 2016): updated sparse besd format; updated features to make sparse verison of BESD; added features to query eQTL summary results; added features to combine BESD files.

6. Version 0.620 (12 Aril, 2016): added a feature to deal with duplicate IDs.

7. Version 0.628 (11 May, 2016): added a feature to visualize SMR results.

8. Version 0.630 (23 May, 2016): updated features to make binary besd file from plain text file(s).

9. Version 0.631 (23 June, 2016): more options to make BESD files and more memory-efficient when making binary besd files.

10. Version 0.632 (28 June, 2016): added a feature to make a BESD file from BOLT-LMM output format.

11. Version 0.64 (8 August, 2016): updated the .esi file format; updated the HEIDI test (a new method that improves the power of the HEIDI test); updated the SMR query output format; improved the analysis to combine multiple BESD files.

12. Version 0.65 (12 December, 2016): added a flag (--heidi-mtd) for users to choose the original approach or a new approach for HEIDI test.

13. Version 0.66 (10 January, 2017): updated the function to generate the file for locus plot. The new version is able to read a gene list with/without strand information.

14. Version 0.67 (22 June, 2017): updated the functions to make BESD file by the following strategy: 1) Z* from N(0, 1) given the p-value. 2) SE* = b / Z* . 3) store b and SE* in BESD. This adjustment guarantees that the re-computed p-value from b and SE being exact the same as the original p-value, useful for data with small sample size.

15. Version 0.68 (11 August, 2017): updated the SMR and HEIDI tests in the trans regions (the previous version focuses only on the top trans-eQTL locus and the new version will run the tests for all the trans-eQTL loci one at a time).

16. (12 September, 2017): Luke R. Lloyd-Jones et al. released CAGE eQTL summary statistics for SMR analysis.

17. Version 0.69 (7 October, 2017): added features to run multi-SNP based SMR and SMR analysis of two molecular traits. Also add a feature to remove technical eQTLs.

Basic options

SMR

 # run SMR and HEIDI test

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --out mysmr --thread-num 10
```

--bfile reads individual-level SNP genotype data (in PLINK binary format) from a reference sample for LD estimation, i.e. .bed, .bim, and .fam files.

--gwas-summary reads summary-level data from GWAS. The input format follows that for GCTA-COJO analysis (http://cnsgenomics.com/software/gcta/#Conditionalanalysis).

mygwas.ma

```
SNP    A1  A2  freq    b    se  p    n
rs1001    A   G   0.8493  0.0024  0.0055  0.6653  129850
rs1002    C   G   0.03606 0.0034  0.0115  0.7659  129799
rs1003    A   C   0.5128  0.045   0.038   0.2319  129830
......
```

Columns are SNP, the effect (coded) allele, the other allele, frequency of the effect allele, effect size, standard error, p-value and sample size. The headers are not keywords and will be omitted by the program. Important: “A1” needs to be the effect allele with “A2” being the other allele and “freq” needs to be the frequency of “A1”. **NOTE:1) For a case-control study, the effect size should be log(odds ratio) with its corresponding standard error**.

--beqtl-summary reads summary-level data from a eQTL study in binary format. We store eQTL summary data in three separate files .esi (SNP information, in the same format as the PLINK .bim file), .epi (probe information) and .besd (eQTL summary statistics in binary format). See Data Management for more information. We have prepared the data from the Westra study (Westra et al. 2013 Nat Genet) in this format, which is available for download at Download.

--out saves the results from the SMR analysis in .smr file (text format).

mysmr.smr

```
ProbeID    Probe_Chr   Gene    Probe_bp    SNP SNP_Chr SNP_bp  A1  A2  Freq    b_GWAS  se_GWAS p_GWAS  b_eQTL  se_eQTL p_eQTL  b_SMR   se_S
MR  p_SMR   p_HEIDI nsnp_HEIDI
prb01    1   Gene1   1001    rs01    1   1011    C   T   0.95    -0.024  0.0063  1.4e-04 0.36    0.048   6.4e-14 -0.0668 0.0197  6.8e-04 NA
  NA
prb02    1   Gene2   2001    rs02    1   2011    G   C   0.0747  0.0034  0.0062  5.8e-01 0.62    0.0396  2e-55   0.0055  0.01    5.8e-01 4.
17e-01    28
......
```

Columns are probe ID, probe chromosome, gene name, probe position, SNP name,SNP chromosome, SNP position, the effect (coded) allele, the other allele, frequency of the effect allele (estimated from the reference samples), effect size from GWAS, SE from GWAS, p-value from GWAS, effect size from eQTL study, SE from eQTL study, p-value from eQTL study, effect size from SMR, SE from SMR, p-value from SMR, p-value from HEIDI (HEterogeneity In Depedent Instruments) test, and number of SNPs used in the HEIDI test.

Missing Value is represented by "NA".

--thread-num specifies the number of OpenMP threads for parallel computing. The default value is 1.

 # Specify a method for HEIDI test

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --heidi-mtd 0 --out mysmr
```

--heidi-mtd specifies a method for HEIDI test. 0 for the original HEIDI test approach as in Zhu et al. (2016 Nature Genetics), and 1 for a new HEIDI test (beta version for testing). The default value is 1. The new approach uses up to the top 20 SNPs in the cis-eQTL region (including the top cis-eQTL) for heterogeneity test because our latest simulation shows that the power of HEIDI test increases initially but then decreases with increasing number of SNPs (m) with a peak at m = ~20.

 # Filter SNPs by MAF (in the reference sample)

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --maf 0.01 --out mysmr
```

--maf removes SNPs based on a minor allele frequency (MAF) threshold in the reference sample.

 # Include or exclude a subset of individuals

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --keep myindi.list --out mysmr
```

--keep includes a subset of individuals in the reference sample for analysis.

--remove excludes a subset of individuals in the reference sample from the analysis.

myindi.list

```
F001 S001
F002 S002
F003 S001
...
```

 # Include or exclude a subset of eQTL summary data

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --extract-snp mysnp.list --extract-probe myprobe.list --out mysmr
```

--extract-snp extracts a subset of SNPs for analysis.

--exclude-snp excludes a subset of SNPs from analysis.

mysnp.list

```
rs1001
rs1002
rs1003
...
```

--extract-probe extracts a subset of probes for analysis.

--exclude-probe excludes a subset of probes from analysis.

myprobe.list

```
probe1001
probe1002
probe1003
...
```

 # Other parameters

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --peqtl-smr 5e-8 --ld-pruning 0.9 --peqtl-heidi 1.57e-3 --heidi-m 3 --ci
s-wind 2000 --thread-num 5 --out mysmr
```

--peqtl-smr p-value threshold to select the top associated eQTL for the SMR test. The default value is 5.0e-8. By default, we only run the SMR analysis in the cis regions. Please see below for the SMR analysis in trans regions.

--peqtl-heidi threshold of eQTL p-value to select eQTLs for the HEIDI test. The default value is 1.57e-3, which is equivalent to a chi-squared value (df=1) of 10.

--ld-pruning LD r-squared threshold for pruning SNPs (eQTLs) in HEIDI test, removing SNPs in high LD with the top associated eQTL. The default value is 0.9.

--heidi-m minimum requirement of the number of eQTLs used in the HEIDI test. We will skip the HEIDI test if the number of SNPs is smaller than the threshold. This is because if the number of SNPs is too small, HEIDI test has little power to detect heterogeneity and possibly generates misleading result. The default value is 3.

--cis-wind defines a window centred around the probe to select cis-eQTLs (passing a p-value threshold) for the SMR analysis. The default value is 2000Kb.

 # Specify a target SNP for the SMR and HEIDI tests

By default, we use the top cis-eQTL as a target in the SMR analysis, i.e. using the top cis-eQTL in the SMR test and then using the top cis-eQTL to test against the other cis-eQTLs in the region for heterogeneity in the HEIDI test. You can also specific the target by the following option. Note that this option will ignore p-value specified by the --peqtl-smr option (--peqtl-heidi still applies).

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --target-snp rs12345 --out mysmr
```

--target-snp specifies a SNP as the target for the SMR and HEIDI tests as described above.

 # Turn off the HEIDI test

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --heidi-off --out mysmr
```

--heidi-off turns off the HEIDI test.

SMR and HEIDI tests in trans regions

The trans-eQTLs are defined as the eQTLs that are more than 5Mb away from the probe.

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --out mytrans --trans --trans-wind 1000
```

--trans turns on SMR and HEIDI tests in trans regions.

--trans-wind defines a window centred around the top associated trans-eQTL to select SNPs (passing a p-value threshold) for the SMR and HEIDI test. The default value is 1000Kb.

mytrans.smr

```
ProbeID    Probe_Chr   Gene    Probe_bp    trans_chr   trans_leftBound trans_rightBound    SNP SNP_Chr SNP_bp  A1  A2  Freq    b_GWAS  se_G
WAS p_GWAS  b_eQTL  se_eQTL p_eQTL  b_SMR   se_SMR  p_SMR   p_HEIDI nsnp_HEIDI
prb01    1   Gene1   1001    16  5349752 7350902 rs01    16  6349942 C   T   0.131   0.0021  0.0152  8.8e-01 -0.214  0.038   3.26e-08
-0.0098 0.071   8.9e-01 1.73e-1 19
prb01    1   Gene1   1001    21  6443018 8459725 rs02    21  7460164 G   C   0.0747  0.0034  0.0062  5.8e-01 0.62    0.0396  2e-55   0.0055
  0.01    5.8e-01 4.17e-01    8
......
```

Columns are probe ID, probe chromosome, gene name, probe position, tans-eQTL chromosome, left boundary of the trans-region, right boundary of the trans-region, SNP name, SNP chromosome, SNP position, the effect (coded) allele, the other allele, frequency of the effect allele (estimated from the reference samples), effect size from GWAS, SE from GWAS, p-value from GWAS, effect size from eQTL study, SE from eQTL study, p-value from eQTL study, effect size from SMR, SE from SMR, p-value from SMR, p-value from HEIDI test, and number of SNPs used in the HEIDI test.

Multi-SNP based SMR test

Below shows an option to combine the information from all the SNPs in a region that pass a p-value threshold (the default value is 5.0e-8 which can be modified by the flag **--peqtl-smr**) to conduct a set-based SMR analysis.

The SNPs are pruned for LD using a weighted vertex coverage algorithm with a LD r2 threshold (the default value is 0.9 which can be modified by the flag **--ld-pruning**) and eQTL p-value as the weight.

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --out mymulti --smr-multi
```

--smr-multi turns on set-based SMR test in the cis-region.

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --out mymulti --smr-multi --set-wind 500
```

--set-wind defines a window width (Kb) centred around the top associated cis-eQTL to select SNPs in the cis-region. The default value is -9 resulting in selecting SNPs in the whole cis-region if this option is not specified.

SMR analysis of two molecular traits

Here we provide an option to test the pleotropic association between two molecular traits using summary data. Take the analysis of DNA methylation and gene expression data as an example. In this case, we will need mQTL and eQTL summary data in BESD format.

```
smr --bfile mydata --beqtl-summary myexposure --beqtl-summary myoutcome  --out myomics
```

--beqtl-summary the first one reads mQTL summary data as the exposure. The second one reads eQTL summary data from as the outcome.

```
smr --bfile mydata --beqtl-summary myexposure --beqtl-summary myoutcome --extract-exposure-probe myeprobein.list --out myomics
```

--extract-exposure-probe extracts a subset of exposure probes for analysis.

--extract-outcome-probe extracts a subset of outcome probes for analysis.

--exclude-exposure-probe excludes a subset of exposure probes from analysis.

--exclude-outcome-probe excludes a subset of outcome probes from analysis.

```
smr --bfile mydata --beqtl-summary myexposure --beqtl-summary myoutcome --extract-single-exposure-probe eprobe1 --extract-single-outcome-pr
obe oprobe1 --out myomics
```

--extract-single-exposure-probe extracts a single exposure probe for analysis.

--extract-single-outcome-probe extracts a single outcome probe for analysis.

```
smr --bfile mydata --beqtl-summary myexposure --beqtl-summary myoutcome --exclude-single-exposure-probe eprobe1 --exclude-single-outcome-pr
obe oprobe1 --out myomics
```

--exclude-single-outcome-probe excludes a single outcome probe from analysis.

--exclude-single-exposure-probe excludes a single exposure probe from analysis.

Data Management

eQTL summary data are usually generated from association tools such as PLINK and stored in separate files in text format (usually one file for each probe) with a very large file size in total. Here we provide an efficient way to store the eQTL summary data in binary format (BESD), with flexible options to query the data for any subset of SNPs and/or probes (see Query eQTL Results). We further provide a sparse version of the BESD format (used as default), which is extremely storage-efficient without losing too much information. The basic idea is that we store summary data of all SNPs within 2Mb of a probe in either direction, all SNPs within 1Mb of any trans-eQTL in either direction, and all SNPs with p < 1e-5 in the rest of the genome (note that all these parameters can be specified by users). We also provide several options to import data in various of formats (e.g. PLINK, GEMMA, BOLT-LMM and other text formats).

BESD format

 # BESD format: an efficient format to store eQTL summary data

We store eQTL summary data in three separate files .esi (SNP information, similar as a PLINK .bim file), .epi (probe information) and .besd (a binary file to store the summary statistics).

myeqtl.esi

```
1    rs1001  0   744055  A   G   0.23
1    rs1002  0   765522  C   G   0.06
1    rs1003  0   995669  T   C   0.11
......
```

Columns are chromosome, SNP, genetic distance (can be any arbitary value since it will not be used in the SMR analysis), basepair position, the effect (coded) allele, the other allele and frequency of the effect allele. ***myeqtl.epi***

```
1    probe1001   0   924243  Gene01  +
1    probe1002   0   939564  Gene02  -
1    probe1003   0   1130681 Gene03  -
......
```

Columns are chromosome, probe ID(can be the ID of an exon or a transcript for RNA-seq data), genetic distance (can be any arbitary value), physical position, gene ID and gene orientation (this information will only be used for graphic presentation, please see [SMR plot]).

myeqtl.besd

eQTL summary-level statistics (effect size and SE) in binary format. Please do not try to open this file with a text editor.

Given the large numbers of SNPs and probes, the size of a .besd file will still be very large. Since the eQTL association signals are highly enriched in the cis-region and often there are not many trans-eQTLs, we could reduce the size of the .besd file by orders of magnitude if we only store the data for SNPs within 2Mb of a probe in either direction, SNPs within 1Mb of any trans-eQTL in either direction, and SNPs with p < 1e-5 in the rest of the genome (see below for options to change these paramters). We call this the sparse BESD format.

We only store effect size (b) and SE in the BESD file, and re-calculate p-value for analysis when necessary, assuming b / SE follows a standard normal distribution, i.e. N(0, 1). Strictly speaking, b / SE follows a t-distribution which is approximately N(0, 1) if sample size is large. For data sets with sample sizes (e.g. GTEx), this might lead to a bias in p-value. We therefore compute z* based on the original p-value from a standard normal distribution, and adjust the standard error as SE = b / z*. This adjustment guarantees that the re-computed p-value from b and SE being exact the same as the original p-value.

See below for options to make a BESD file from data in several different formats.

Make a BESD file

 # Make a BESD file from eQTL summary data in ESD format

To compile data in sparse BESD format

```
smr --eqtl-flist my.flist --make-besd --out mybesd
```

--eqtl-flist reads a file to get probe information and file paths of the eQTL summary data.

--make-besd saves summary data in BESD format. By default, the data will be stored in sparse BESD format (See below for the option **--make-besd-dense** to store the data in dense BESD format). By default, the data will be stored in sparse BESD format if the sparsity given the parameters (by default, ±2Mb of the cis-region, ±1Mb of any trans-eQTL and all SNP at p < 1e-5) is lower than 0.4. It will also output a text file (.summary) to summarise the genomic regions stored in the .besd file (sparse format) for each probe.

my.flist

```
Chr    ProbeID GeneticDistance ProbeBp Gene    Orientation PathOfEsd
9    cg00000658  0   139997924   MAN1B1  -   path/my01.esd
```

```
20    cg26036652  0   33735834    NA  NA  path/my02.esd
1    cg00489772  0   3775078 NA  NA  path/my03.esd
......
```

This is a text file **with headers**. The first 6 columns are the same as in .epi. The last column is the full path of an eQTL summary data file (.esd file, see below for the format of a .esd file).

my01.esd

```
Chr    SNP Bp  A1  A2  Freq    Beta    se  p
9    rs12349815  150048  T   A   0.968   0.019   0.016   0.2434
20    rs141129176 62955484    G   A   0.89    0.012   0.009   0.2156
......
```

This is a text file **with headers**. Columns are chromosome, SNP, the effect (coded) allele, the other allele, basepair position, frequency of the effect allele, effect size, standard error and p-value.

HINT : if the SNPs in all of the .esd files are identical, the efficiency of the analysis can be largely improved by adding the --geno-uni option. This option call be used in all the commands of this section.

```
smr --eqtl-flist my.flist --make-besd --geno-uni --out mybesd
```

--geno-uni indicates all the input .esd files are identical.

To compile eQTL summary data in sparse BESD format with user-specified parameters

```
smr --eqtl-flist my.flist --make-besd  --cis-wind 2000 --trans-wind 1000 --peqtl-trans 5.0e-8 --peqtl-other 1.0e-5 --out mybesd
```

--cis-wind specifies a window (in Kb unit) to store all the SNPs within the window of the probe in either direction. The default value is 2000Kb.

--trans-wind specifies a window (in Kb unit) to store all the SNPs in a trans-region. If there is a trans-eQTL with p-value < the specified threshold (--peqtl-trans), it will store all the SNPs within the window of the top associated trans-eQTL in either direction. The default value is 1000Kb.

--peqtl-trans p-value threshold for trans-eQTLs. The default value is 5.0e-8.

--peqtl-other Apart from the cis and trans regions, it will also store all SNPs with eQTL p-values < this threshold. The default value is 1.0e-5 .

To compile the eQTL summary data in dense BESD format

```
smr --eqtl-flist my.flist --make-besd-dense --out mybesd
```

--make-besd-dense saves summary data of all SNPs for all probes.

WARNING : This will generate a huge file.

NOTE : the **--make-besd-dense** option can be used in all the commands above and below.

 # Make a BESD file from eQTL summary data in PLINK-qassoc output format

The output file from a PLINK --assoc analysis does not contain allele information. We therefore need to read the alleles from a PLINK .bim file. The file path of the PLINK .bim file needs to be added as the last column of the .flist file (see the example below).

```
smr --eqtl-flist my.flist  --plink-qassoc-format --make-besd --out mybesd
```

--plink-qassoc-format reads eQTL summary data in PLINK-qassoc output format (output file from a PLINK --assoc analysis for a quantitative trait).

my.flist

```
Chr    ProbeID GeneticDistance ProbeBp Gene    Orientation PathOfEsd   PathOfBim
9    cg00000658  0   139997924   MAN1B1  -   path_assoc/my01.qassoc  path_genotype/chr9
20    cg26036652  0   33735834    NA  NA  path_assoc/my02.qassoc  path_genotype/chr20
1    cg00489772  0   3775078 NA  NA  path_assoc/my03.qassoc  path_genotype/chr19
......
```

NOTE : The program is able to read *.tar.gz file, e.g. path_assoc/my03.qassoc.tar.gz

 # Make a BESD file from eQTL summary data in GEMMA output format

```
smr --eqtl-flist my.flist --gemma-format --make-besd --out mybesd
```

--gemma-format reads eQTL summary data in GEMMA association output format

```
chr    rs  ps  n_miss  allel1  allel0  af  beta    se  l_remle p_wald
1    rs3683945   3197400 0   A   G   0.443   -7.788665e-02   6.193502e-02    4.317993e+00    2.087616e-01
1    rs3707673   3407393 0   G   A   0.443   -6.654282e-02   6.210234e-02    4.316144e+00    2.841271e-01
1    rs6269442   3492195 0   A   G   0.365   -5.344241e-02   5.377464e-02    4.323611e+00    3.204804e-01
......
```

The 11 columns are: chromosome, SNP ID, basepair position, number of missing values for a given SNP, the effect (coded) allele, the other allele, frequency of the effect allele, effect size, standard error, lambda and p-value ([http://www.xzlab.org/software.html]).

 # Make a BESD file from eQTL summary data in BOLT-LMM output format

```
smr --eqtl-flist my.flist  --bolt-assoc-format --make-besd --out mybesd
```

--bolt-assoc-format reads eQTL summary data in BOLT_LMM output format

```
SNP    CHR BP  GENPOS  ALLELE1 ALLELE0 A1FREQ  F_MISS  BETA    SE  P_BOLT_LMM_INF  P_BOLT_LMM
rs58108140    1   10583   0.000000    A   G   0.109810    0.011935    0.074942    0.045043    9.6E-02 9.7E-02
rs180734498    1   13302   0.000000    T   C   0.061042    0.007595    0.084552    0.058078    1.5E-01 1.4E-01
rs151118460    1   91581   0.000000    A   G   0.399377    0.013382    0.024344    0.034394    4.8E-01 4.8E-01
......
```

The 12 columns are: SNP ID, chromosome, basepair position, genetic position, the effect (coded) allele, the other allele, frequency of the effect allele, fraction of individuals with missing genotype at the SNP, effect size, standard error, infinitesimal model (mixture model) association test p-value, and non-infinitesimal model association test p-value ([https://data.broadinstitute.org/alkesgroup/BOLT-LMM/#x1-440008.1]).

 # Make a BESD file from a single text file (in SMR query output format)

```
smr --qfile myquery.txt --make-besd --out mybesd
```

--qfile reads eQTL summary data in SMR query output format (see Query eQTL Results for the format of a query output file).

 # Make a BESD file from BESD file(s)

To make a sparse BESD file from a single dense BESD file

```
smr --beqtl-summary my_beqtl --make-besd --out my_sparse
```

```
smr --beqtl-summary my_beqtl --cis-wind 2000 --trans-wind 1000 --peqtl-trans 5.0e-8 --peqtl-other 1.0e-5 --make-besd --out my_sparse
```

To make a sparse BESD file from multiple sparse or dense BESD files (can be a mixture of both types)

```
smr --besd-flist my_file.list --make-besd --out my_sparse
```

--besd-flist reads a file to get the full paths of the BESD files.

my_file.list

```
path1/my_besd1
path2/my_besd2
path3/my_besd3
...
```

NOTE : this command can be used to merge multiple BESD files.

HINT : if the SNPs in all the .esi files are identical, you can speed up the analysis using the **--geno-uni** option.

 # Extract or remove a subset of eQTL summary data (subset BESD)

To extract a subset of SNPs and/or probes

```
smr --beqtl-summary myeqtl --extract-snp mysnp.list --extract-probe myprobe.list  --make-besd --out mybesd
```

To remove a subset of SNPs and/or probes

```
smr --beqtl-summary myeqtl --exclude-snp mysnp.list --exclude-probe myprobe.list  --make-besd --out mybesd
```

 # Update frequency of the effect allele

To add or update the frequencies of the effect alleles

```
smr --beqtl-summary myeqtl --update-freq mysnp.freq
```

--update-freq reads an input file with allele frequency information and adds a new column (i.e. frequency the effect allele) to the .esi file.

mysnp.freq

```
rs12349815    T   A   0.968
rs141129176    G   A   0.89
......
```

The input is a text file **without headers**. Columns are SNP, the effect allele, the other allele and frequency of the effect allele.

NOTE : the SMR program is compatibile with .esi files with or without frequency information.

Remove technical eQTLs

 # Remove technical eQTLs

Filtering out eQTLs for which there is a significant cis-eQTL in the hybridization region of the probe. This option will remove all the SNPs in the cis-region of the probe and save the removed data in a file in SMR Query format (see Query eQTL Results for the format of a query output file). The default p-value threshold is 5e-8, which can be changed by the **--p-technical** (see below).

```
smr --beqtl-summary myeqtl --rm-technical probe_hybrid.txt --make-besd --out mybesd
```

--rm-technical specifies the probe hybridization region and excludes the technical eQTLs.

probe_hybrid.txt

```
19    probe0  50310094    50310143
19    probe1  406496  406545
10    probe2  119293020   119293069
......
```

This is a text file **without headers**. Columns are chromosome, probe ID, start of the hybridization region and end of the hybridization region.

```
smr --beqtl-summary myeqtl --rm-technical probe_hybrid.txt --p-technical 5e-8 --make-besd --out mybesd
```

--p-technical reads a p-value threshold to select technical eQTLs. The default value is 5e-8.

Extract cis-regions

 # Extract cis-regions of eQTL summary data

```
smr --beqtl-summary myeqtl --extract-cis --make-besd --out mybesd
```

--extract-cis extracts the cis-eQTL summary data.

SMR locus plot

Visualization of SMR results

Here we provide an R script to plot SMR results as presented in Zhu et al. (2016 Nature Genetics). The data file for plot can be generated by the command below. The R script is avaliable at Download.

 # SMR command line to generate a data file for plot

```
smr --bfile mydata --gwas-summary mygwas.ma --beqtl-summary myeqtl --out myplot --plot --probe ILMN_123 --probe-wind 500 --gene-list glist-hg19
```

--plot saves the data file for plot in text format .

--gene-list specifies a gene range list.

glist-hg19 (without strand information)

```
19    58858171    58864865    A1BG
19    58863335    58866549    A1BG-AS1
10    52559168    52645435    A1CF
......
```

This is a text file without headers. The columns are chromosome code, start of gene, end of gene and gene ID.

The gene range lists (mirrored from [PLINK2] website) are

- **hg18:** glist-hg18 (older, ASCII-sorted PNGU version)
- **hg19:** glist-hg19
- **hg38:** glist-hg38

Not all the genes in the region but only those in the .epi file will be drawn in the locus plot since the strand information is absent from the gene list provied in the PLINK2 website. To fix this problem, we updated SMR to read a gene list with/without strand information.

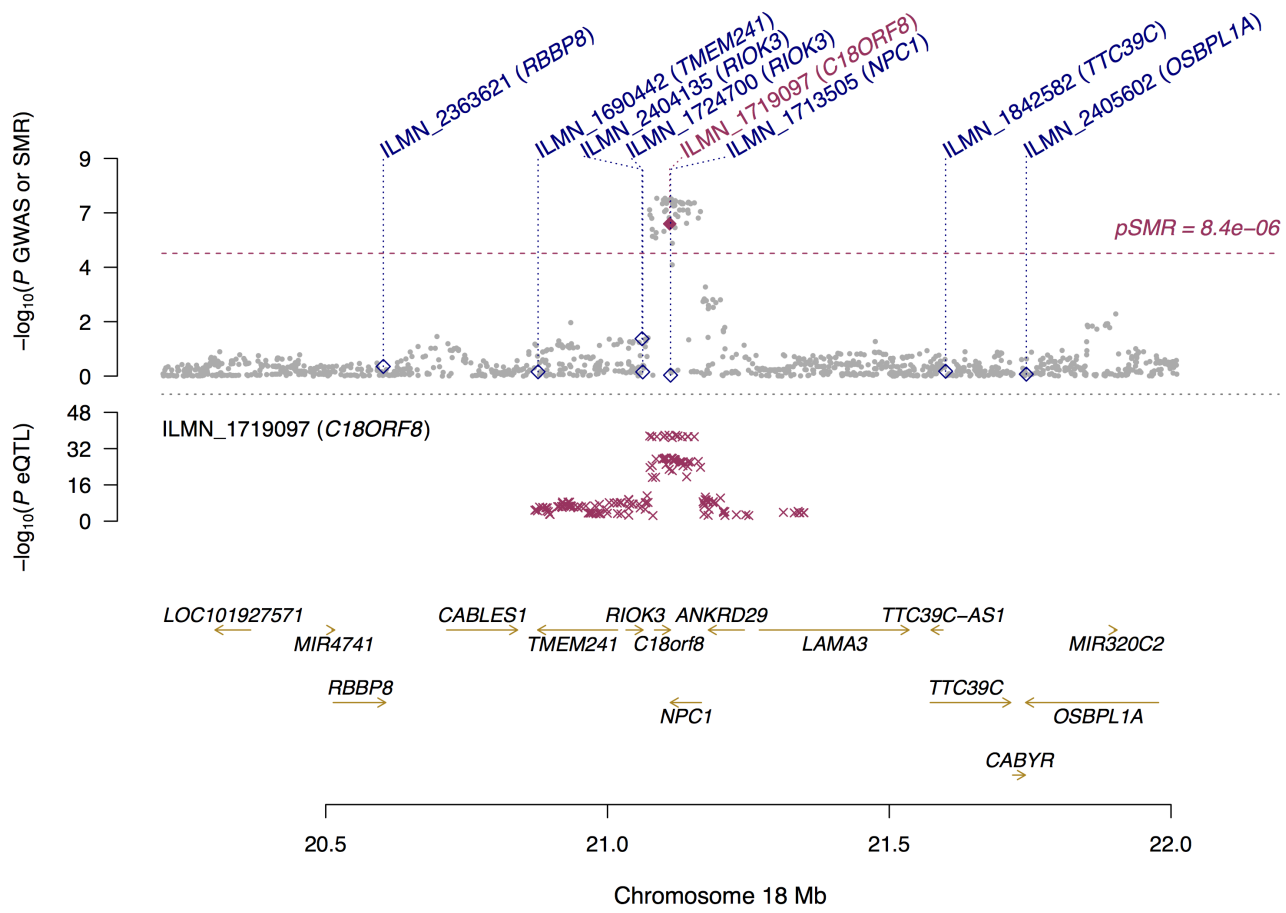
glist-hg19 (with strand information)

```
19    58858171    58864865    A1BG    -
19    58863335    58866549    A1BG-AS1    +
10    52559168    52645435    A1CF    -
......
```

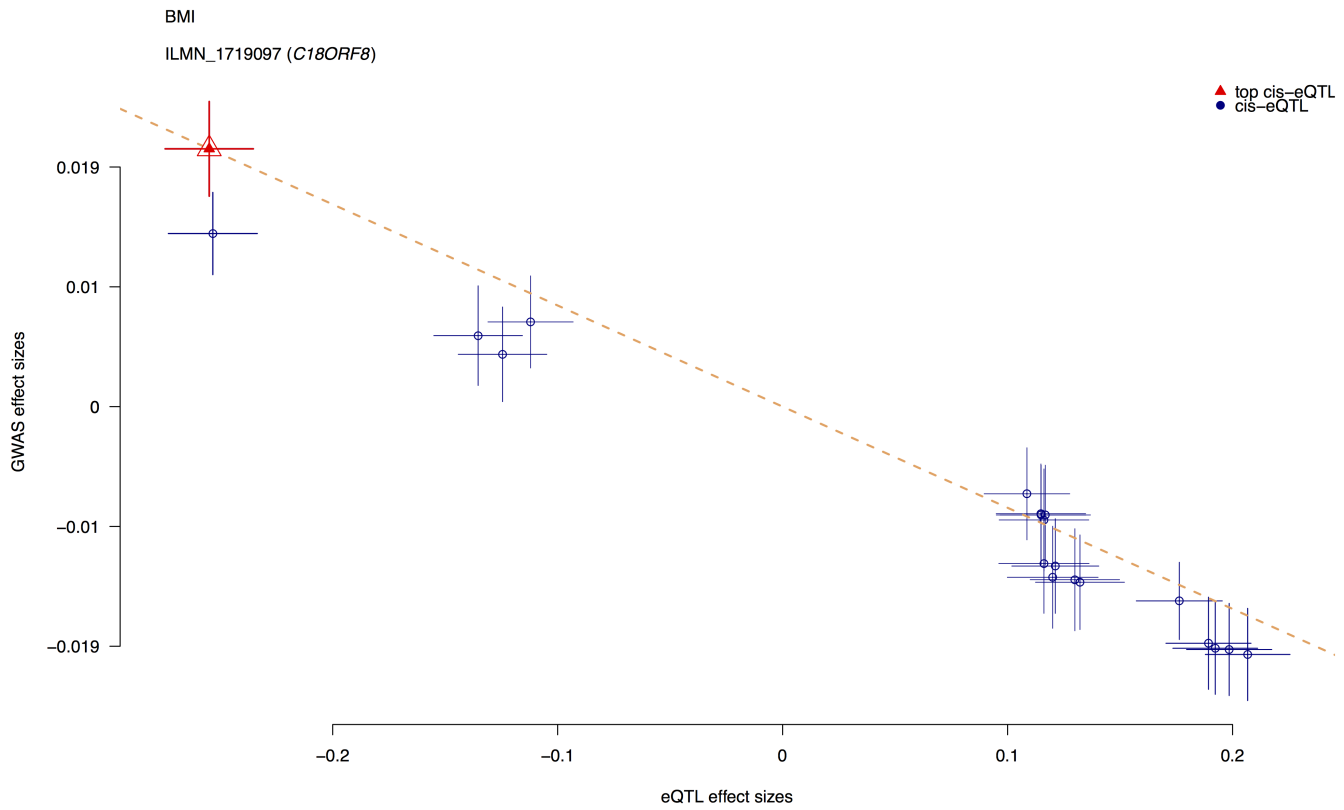
This is a text file without headers. The columns are chromosome code, start of gene, end of gene, gene ID and gene strand.

 # R commands to draw the plots

```
source("plot_SMR.r")
# Read the data file in R:
SMRData = ReadSMRData("myplot.ILMN_123.txt")
# Plot the SMR results in a genomic region centred around a probe:
SMRLocusPlot(data=SMRData, smr_thresh=8.4e-6, heidi_thresh=0.05, plotWindow=1000, max_anno_probe=16)
# smr_thresh: genome-wide significance level for the SMR test.
# heidi_thresh: threshold for the HEIDI test. The default value is 0.05.
# cis_wind: size of a window centred around the probe to select cis-eQTLs for plot. The default value is 2000Kb.
# max_anno_probe: maximum number of probe names to be displayed on the figure. The default value is 16.
```




```
# Plot effect sizes from GWAS against those from eQTL study:
SMREffectPlot(data=SMRData, trait_name="BMI")
# trait_name: name of the trait or disease.
```



Query eQTL Results

Query eQTL Summary Results

Since the eQTL summary are stored in binary format for a large number of probes and SNPs, we provide the options below to query the eQTL summary results from command line options or from file-list options given a specified eQTL p-value threshold.

 # Command line options for SNPs

To query the eQTL resutls for a single SNP, we could use this command

```
smr --beqtl-summary myeqtl --query 5.0e-8 --snp rs123 --out myquery
```

--query saves in text format a subset of the eQTL summary dataset based on the specified eQTL p-value threshold. The default value is 5.0e-8.

--snp specifies a single SNP.

myquery.txt

```
SNP    Chr BP  A1  A2  Freq    Probe   Probe_Chr   Probe_bp    Gene    Orientation b   se  p
rs01    1   1001    A   G   0.23    cg01    1   1101    gene1   +   −0.033  0.006   3.8e−08
rs01    1   1001    A   G   0.06    cg02    1   1201    gene2   −   0.043   0.007   8.1e−10
......
```

To query eQTL resutls for a range of SNPs in a genomic region

```
smr --beqtl-summary myeqtl --query 5.0e-8 --from-snp rs123 --to-snp rs456 --out myquery
```

--from-snp specifies the start SNP.

--to-snp specifies the end SNP.

NOTE : All SNPs should be on the same chromosome.

To query eQTL results for all SNP on a chromosome

```
smr --beqtl-summary myeqtl --query 5.0e-8 --snp-chr 1
```

--snp-chr specifies a chromosome to select SNPs.

NOTE : The probes in the result could be on the other chromosomes if there are *trans*-eQTLs.

To query SNPs based on physical positions

```
smr --beqtl-summary myeqtl --query 5.0e-8 --snp-chr 1 --from-snp-kb 100 --to-snp-kb 200 --out myquery
```

--from-snp-kb specifies the start physical position of the region.

--to-snp-kb specifies the end physical position of the region.

NOTE : You will need to specify a chromosome (using the '--snp-chr' option) when using this option.

To query based on a flanking region of a SNP

```
smr --beqtl-summary myeqtl --query 5.0e-8 --snp rs123 --snp-wind 50 --out myquery
```

--snp-wind defines a window centred on a specified SNP.

 # Command line options for probes

To query based on a single probe

```
smr --beqtl-summary myeqtl --query 5.0e-8 --probe cg123 --out myquery
```

--probe specifies a single probe.

To query based on a range of probes

```
smr --beqtl-summary myeqtl --query 5.0e-8 --from-probe cg123 --to-probe cg456 --out myquery
```

--from-probe specifies the start probe.

--to-probe specifies the end probe.

NOTE : All probes should be on the same chromosome.

To query based on a chromosome

```
smr --beqtl-summary myeqtl --query 5.0e-8 --probe-chr 1
```

--probe-chr specifies a chromosome to select probes.

NOTE : The SNPs in the result could be on the other chromosomes if there are *trans*-eQTLs.

To query based on physical positions of the probes

```
smr --beqtl-summary myeqtl --query 5.0e-8 --probe-chr 1 --from-probe-kb 1000 --to-probe-kb 2000 --out myquery
```

--from-probe-kb specifies the start physical position of the probes.

--to-probe-kb specifies the end physical position of the probes.

NOTE : You will need to specify a chromosome (using the '--probe-chr' option) when using this option.

To query based on a flanking region of a probe

```
smr --beqtl-summary myeqtl --query 5.0e-8 --probe cg123 --probe-wind 1000 --out myquery
```

--probe-wind defines a window centred on a specified probe.

To query based on a gene

```
smr --beqtl-summary myeqtl --query 5.0e-8 --gene gene1 --out myquery
```

--gene specifies a single gene to select probes.

 # Command line option for cis-region

```
smr --beqtl-summary myeqtl --query 5.0e-8 --probe cg123 --cis-wind 2000 --out myquery
```

 # File-list options

To query based on a list of SNPs

```
smr --beqtl-summary myeqtl --extract-snp snp.list --query 5.0e-8 --out myquery
```

To query based on a list of probes

```
smr --beqtl-summary myeqtl --extract-probe probe.list --query 5.0e-8 --out myquery
```

To qurey based on a list of genes

```
smr --beqtl-summary myeqtl --genes gene.list --query 5.0e-8 --out myquery
```

--genes extracts a subset of probes which tag the genes in the list.

gene.list

```
gene1
gene2
gene3
...
```

MeCS

 # Overview

MeCS is a method that only requires summary-level cis-eQTL data to perform a meta-analysis of cis-eQTLs from multiple cohorts (or tissues) with sample overlaps. It estimates the proportion of sample overlap from null SNPs in the cis-regions and meta-analysed the eQTL effects using a generalized least squares approach. The method can be applied to data from genetic studies of molecular phenotypes (e.g. DNA methylation and histone modification).

Bug reports or questions to Jian Yang (jian.yang@uq.edu.au) at Institute for Molecular Bioscience, The University of Queensland.

 # Citation

Qi et al. Identifying gene targets for brain-related traits using transcriptomic and methylomic data from blood. *Submitted*.

 # Tutorial

> Example

```
smr --besd-flist my_file.list --mecs --thread-num 5 --out mecs_result
```

--mecs implements the MeCS analysis.

--meta implements the conventional inverse-variance-weighted meta-analysis assuming all the cohorts are independent.

> Example

```
smr --besd-flist my_file.list --meta --thread-num 5 --out meta_result
```

Options Reference

Option	Description
--beqtl-flist	reads a file to get the full paths of the BESD files.
--beqtl-summary	reads summary-level data from a eQTL study in binary format.
--bfile	reads individual-level SNP genotype data in PLINK binary format.
--bolt-assoc-format	reads eQTL summary data in BOLT_LMM output format.
--cis-wind	defines a window centred around the probe to select cis-eQTLs.
--eqtl-flist	reads a file to get probe information and locations of the eQTL summary data files.
--exclude-exposure-probe	excludes a subset of exposure probes from analysis.
--exclude-outcome-probe	excludes a subset of outcome probes from analysis.
--exclude-probe	excludes a subset of probes from analysis.
--exclude-single-exposure-probe	excludes a single exposure probe from analysis.
--exclude-single-outcome-probe	excludes a single outcome probe from analysis.
--exclude-snp	excludes a subset of SNPs from analysis.
--extract-cis	extracts the cis-eQTLs.
--extract-exposure-probe	extracts a subset of exposure probes for analysis.
--extract-outcome-probe	extracts a subset of outcome probes for analysis.
--extract-probe	extracts a subset of probes for analysis.
--extract-single-exposure-probe	extracts a single exposure probe for analysis.
--extract-single-outcome-probe	extracts a single outcome probe for analysis.
--extract-snp	extracts a subset of SNPs for analysis.
--from-probe	specifies the start probe.
--from-probe-kb	specifies the start physical position of the probes.
--from-snp	specifies the start SNP.
--from-snp-kb	specifies the start physical position of the region.
--gemma-fomat	reads eQTL summary data in GEMMA association output format.
--gene	specifies a single gene to select probes.
--genes	extracts a subset of probes which tag the genes in the list.
--gene-list	specifies a gene annotation file.

--geno-uni	indicates all the input .esd files are identical.
--gwas-summary	reads summary-level data from GWAS in GCTA-COJO format.
--heidi-m	minimum requirement of the number of eQTLs used in the HEIDI test.
--heidi-mtd	specify a method for HEIDI test.
--heidi-off	turns off the HEIDI test.
--keep	includes a subset of individuals in the reference sample for analysis.
--ld-pruning	LD r-squared threshold for pruning SNPs (eQTLs) in HEIDI test, removing SNPs in high LD with the top associated eQTL.
--maf	removes SNPs based on a minor allele frequency (MAF) threshold in the reference sample.
--make-besd	saves summary data in BESD format. By default, the data will be stored in sparse BESD format.
--make-besd-dense	saves summary data of all SNPs for all probes.
--out	specifies filename prefix for output files.
--p-technical	reads a p-value threshold to select technical eQTLs.
--peqtl-heidi	threshold of eQTL p-value to select eQTLs for the HEIDI test.
--peqtl-other	threshold of eQTL p-value to select eQTLs apart from the cis and trans regions.
--peqtl-smr	threshold of eQTL p-value to select the top associated eQTL for the SMR test.
--peqtl-trans	threshold of eQTL p-value for trans-SNPs.
--plink-qassoc-fomat	reads eQTL summary data in PLINK-qassoc format.
--plot	saves in text format the data for plot.
--probe	specifies a single probe.
--probe-chr	specifies a chromosome to select probes.
--probe-wind	defines a window centred on a specified probe.
--qfile	reads eQTL summary data in SMR query output format.
--query	saves in text format a subset of the eQTL summary dataset based on the specified eQTL p-value threshold.
--remove	excludes a subset of individuals in the reference sample from the analysis.
--rm-technical	specifies the probe hybridization region and excludes the technical eQTLs.
--set-wind	defines a window width (Kb) centred around the top associated cis-eQTL to select SNPs in the cis-region.
--smr-multi	turns on set-based SMR test in the cis-region.
--snp	specifies a single SNP.
--snp-chr	specifies a chromosome to select SNPs.
--to-probe	specifies the end probe.
--to-snp	specifies the end SNP.
--snp-wind	defines a window centred on a specified SNP.
--to-probe-kb	specifies the end physical position of the probes.
--to-snp-kb	specifies the end physical position of the region.
--trans	turns on SMR and HEIDI tests in trans regions.
--trans-wind	defines a window centred around the top associated trans-eQTL to select SNPs .
--target-snp	specifies a SNP as the target for the SMR and HEIDI tests as described above.
--thread-num	specifies the number of OpenMP threads for parallel computing.
--update-freq	reads allele frequency file.