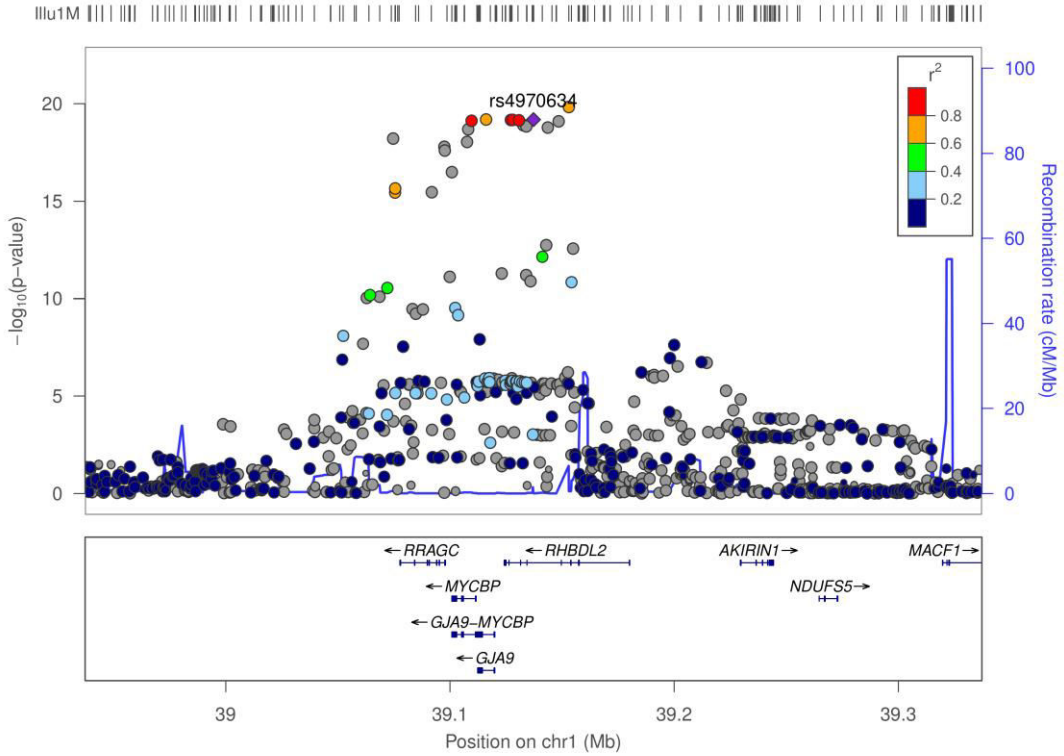
FM-pipeline

This is a pipeline for finemapping using GWAS summary statistics, implemented in Bash as a series of steps to furnish an incremental analysis. As depicted in the diagram below



LocusZoom plot showing Regional association for chr1:39114617-39614617

where our lead SNP rs4970634 is in LD with many others, the procedure attempts to identify causal variants from region(s) showing significant SNP-trait association.

The process involves the following steps, 1. Extraction of effect (beta)/z statistics from GWAS summary statistics (.sumstats), 2. Extraction of correlation from the reference panel among overlapped SNPs from 1 and the reference panel containing individual level data. 3. Information from 1 and 2 above is then used as input for finemapping.

The measure of evidence is typically (log10) Bayes factor (BF) and associate SNP probability in the causal set.

Software included in this pipeline are listed in the table below.

Name	Function	Input	Output	Reference
CAVIAR	finemapping	z, correlation matrix	causal sets and probabilities	Hormozdiari, et al. (2014)
CAVIARBF	finemapping	z,	BF and	Chen, et al.

		correlation matrix	probabilities for all configurations	(2015)
GCTA	joint/conditional analysis	.sumstats, reference data	association results	Yang, et al. (2012)
FM-summary	finemapping	.sumstats association results	updated results	Huang, et al. (2017)
JAM	finemapping	beta, individual reference data	Bayes Factor of being causal	Newcombe, et al. (2016)
LocusZoom	regional plot	partial .sumstats	.pdf/.png plots	Pruim, et al. (2010)
fgwas	functional GWAS			Pickrell (2014)
finemap	finemapping	z, correlation matrix	causal SNPs and configuration	Benner, et al. (2016)

so they range from regional association plots via LocusZoom, joint/conditional analysis via GCTA, functional annotation via fgwas to dedicated finemapping software including CAVIAR, CAVIARBF, an adapted version of FM-summary, R2BGLiMS/JAM and finemap. One can optionally use a subset of these for a particular analysis by specifying relevant flags from the pipeline's settings.

INSTALLATION

On many occasions, the pipeline takes advantage of the GNU parallel.

Besides (sub)set of software listed in the table above, the pipeline requires GTOOL, PLINK 1.9, and the companion program LDstore from finemap's website need to be installed.

The pipeline itself can be installed in the usual way,

```
git clone https://github.com/jinghuazhao/FM-pipeline
```

The setup is in line with summary statistics from consortia where only RSid are given for the fact that their chromosomal position may be changed over different builds. To remedy this, we use information from UCSC, i.e.,

```
wget http://hgdownload.soe.ucsc.edu/goldenPath/hg19/database/snp150.txt.gz
gunzip -c snp150.txt.gz | \
awk '{split($2,a,"_");sub(/chr/,"",a[1]);print a[1],$4,$5}' | \
sort -k3,3 > snp150.txt
```

Note that JAM requires Java 1.8 so call to Java -jar inside the function needs to reflect this, not straightforward with `install_github()` from `devtools` but one needs to clone the package, modify the R source code and then use

```
git clone https://github.com/pjnewcombe/R2BGLiMS
### change java to java-1.8 in R2BGLiMS/R/R2BGLiMS.R
R CMD INSTALL R2BGLiMS
```

Implementations have been done for the finemapping software along with LocusZoom and GCTA; support for fgwas is still alpha tested. To facilitate handling of grapahics, e.g., importing them into Excel, pdftopng from xpdf is used.

USAGE

Before start, settings at the beginning of the script need to be changed and only minor change is expected after this. The syntax of pipeline is then simply

```
bash fmp.sh <input>
```

Inputs

--- GWAS summary statistics and lead SNPs ---

The **first input file** will be GWAS summary statistics with the following columns,

Column	Name	Description
1	SNP	RSid
2	A1	Effect allele
3	A2	Other allele
4	freqA1	A1 frequency
5	beta	effect estimate
6	se	standard error of effect
7	P	P-vale
8	N	sample size
9*	chr	chromosome
10*	pos	position

This format is in line with joint/conditional analysis by GCTA. Note the last two columns are not normally required but can obtained from UCSC.

The **second input file** is a list of SNPs for which finemapping will be conducted.

A header is required for neither file.

--- Reference panel ---

The pipeline uses a reference panel in a .GEN format, taking into account directions of effect in both the GWAS summary statistics and the reference panel. Its development will facilitate

summary statistics from a variety of consortiua as with reference panels such as the HRC and 1000Genomes.

A .GEN file is required for each region, named such that chr{chr}_{start}_{end}.gen, together with a sample file. For our own data, a utility program in Stata is written to generate such files from their whole chromosome counterpart using SNPinfo.dta.gz which has the following information,

chr	rsid	RSnum	pos	FreqA2	info	type	A1	A2
1	1:54591_A_G	rs561234294	54591	.0000783	.33544	0	A	G
1	1:55351_T_A	rs531766459	55351	.0003424	.5033	0	T	A
...	...	...	...	...	...	...	...	...

Given these, one can do away with Stata and work on a text version for instance SNPinfo.txt. When option stbed=1 in the settings, it only generates st.bed which contains chr, start, end, rsid, pos, r corresponding to the lead SNPs specified and r is a sequence number of region. As GCTA conditional/joint analysis requires whole chromosome reference the counterpart is HRC.do. Note both filter SNPs on minor allele count and measure of imputation quality.

Optionally, a file is specified which contains sample to be excluded from the reference panel; one leaves it unspecified when not needed

We illustrate use of 1000Genomes reference panel, available as FUSION LD reference panel, the code to generate `SNPinfo.dta.gz`

```
#2-12-2017 MRC-Epid JHZ

wget -qO- https://data.broadinstitute.org/alkesgroup/FUSION/LDREF.tar.bz2 |
tar xfj - --strip-components=1
seq 22|awk -vp=1000G.EUR. '{print p $1 ".bed " p $1 ".bim " p $1 ".fam"}' >
merge-list
plink-1.9 --merge-list merge-list --make-bed --out EUR
plink-1.9 --bfile EUR --freq --out EUR
awk -vOFS="\t" '(NR>1){print $2,$5}' EUR.frq > EUR.dat
stata <<END
  insheet rsid FreqA2 using EUR.dat, case
  sort rsid
  gzsave EUR, replace
  insheet chr rsid m pos A1 A2 using EUR.bim, case clear
  gen RSnum=rsid
  gen info=1
  gen type=2
  sort rsid
  gzmerge using EUR
  gen
snpid=string(chr)+":"+string(pos,"%12.0f")+"_"+cond(A1<A2,A1,A2)+"_"+cond(A1<
A2,A2,A1)
  sort chr pos
  drop _merge
  gzsave SNPinfo, replace
```

```
END
seq 22|parallel -j4 -C' ' 'plink-1.9 --bfile 1000G.EUR.{} --recode oxford
gen-gz --out chr{}'
```

where we download and extract the data on the fly. The associate p0.do is also given.

Outputs

The output will involve counterpart(s) from individual software, i.e., .set/post, caviarbf, .snp/.config, .jam/.top

Software	Output type	Description
CAVIAR	.set/.post	causal set and probabilities in the causal set/posterior probabilities
CAVIARBF	.caviarbf	causal configurations and their BFs
FM-summary	.txt	additional information to the GWAS summary statistics
JAM	.jam/.top	the posterior summary table and top models containing selected SNPs
finemap	.snp/.config	top SNPs with largest log10(BF) and top configurations as with their log10(BF)

It is helpful to examine directions of effects together with the correlation of them, e.g., for use with finemap, the code here is now embedded in the pipeline as with an example.

EXAMPLE

We show how to set up for BMI GWAS summary data as reported by the GIANT consortium, Locke, et al. (2015),

```
# GWAS summary statistics
wget
http://portals.broadinstitute.org/collaboration/giant/images/1/15/SNP_gwas_mc
_merge_nogc.tbl.uniq.gz
gunzip -c SNP_gwas_mc_merge_nogc.tbl.uniq.gz |
awk 'NR>1' | \
join -11 -23 - snp150.txt | \
awk '($9!="X" && $9!="Un")' > bmi.txt

# A list of 97 SNPs
R --no-save <<END
library(openxlsx)
```

```
xlsx <- "https://www.nature.com/nature/journal/v518/n7538/extref/nature14177-
s2.xlsx"
snps <- read.xlsx(xlsx, sheet = 4, colNames=FALSE, skipEmptyRows = FALSE,
cols = 1, rows = 5:101)
snplist <- sort(as.vector(snps[,1]))
write.table(snplist, file="97.snps", row.names=FALSE, col.names=FALSE,
quote=FALSE)
END
```

where we download the GWAS summary statistics adding SNP positions in build 37 rather than 36. The list of SNPs can also be used to generate st.bed as above.

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SOFTWARE AND REFERENCES

CAVIAR

Hormozdiari F, et al. (2014) Identifying Causal Variants at Loci with Multiple Signals of Association. Genetics, 44, 725–731

CAVIARBF

Chen W, et al. (2015) Fine Mapping Causal Variants with an Approximate Bayesian Method Using Marginal Test Statistics. Genetics 200:719-736.

FM-summary

Huang H, et al (2017) Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature 547, 173–178, doi:10.1038/nature22969

GCTA

Yang J, et al. (2012) Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. Nat Genet 44:369-375

JAM

Newcombe PJ, et al. (2016) JAM: A Scalable Bayesian Framework for Joint Analysis of Marginal SNP Effects. Genet Epidemiol 40:188–201

LocusZoom

Pruim RJ, et al. (2010) LocusZoom: Regional visualization of genome-wide association scan results. Bioinformatics 2010 September 15; 26(18): 2336.2337

fgwas

Pickrell JK (2014) Joint analysis of functional genomic data and genome-wide association studies of 18 human traits. bioRxiv 10.1101/000752

finemap

Benner C, et al. (2016) FINEMAP: Efficient variable selection using summary data from genome-wide association studies. Bioinformatics 32, 1493-1501

Benner C, et al. (2017) Prospects of Fine-Mapping Trait-Associated Genomic Regions by Using Summary Statistics from Genome-wide Association Studies. Am J Hum Genet 101(4):539-551

GIANT paper

Locke AE, et al. (2015) Genetic studies of body mass index yield new insights for obesity biology. Nature 518(7538):197-206. doi: 10.1038/nature14177