Practical Session 8

Sequence-based Association, Interpretation, Visualization using EPACTS

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STOM 2014 Workshop

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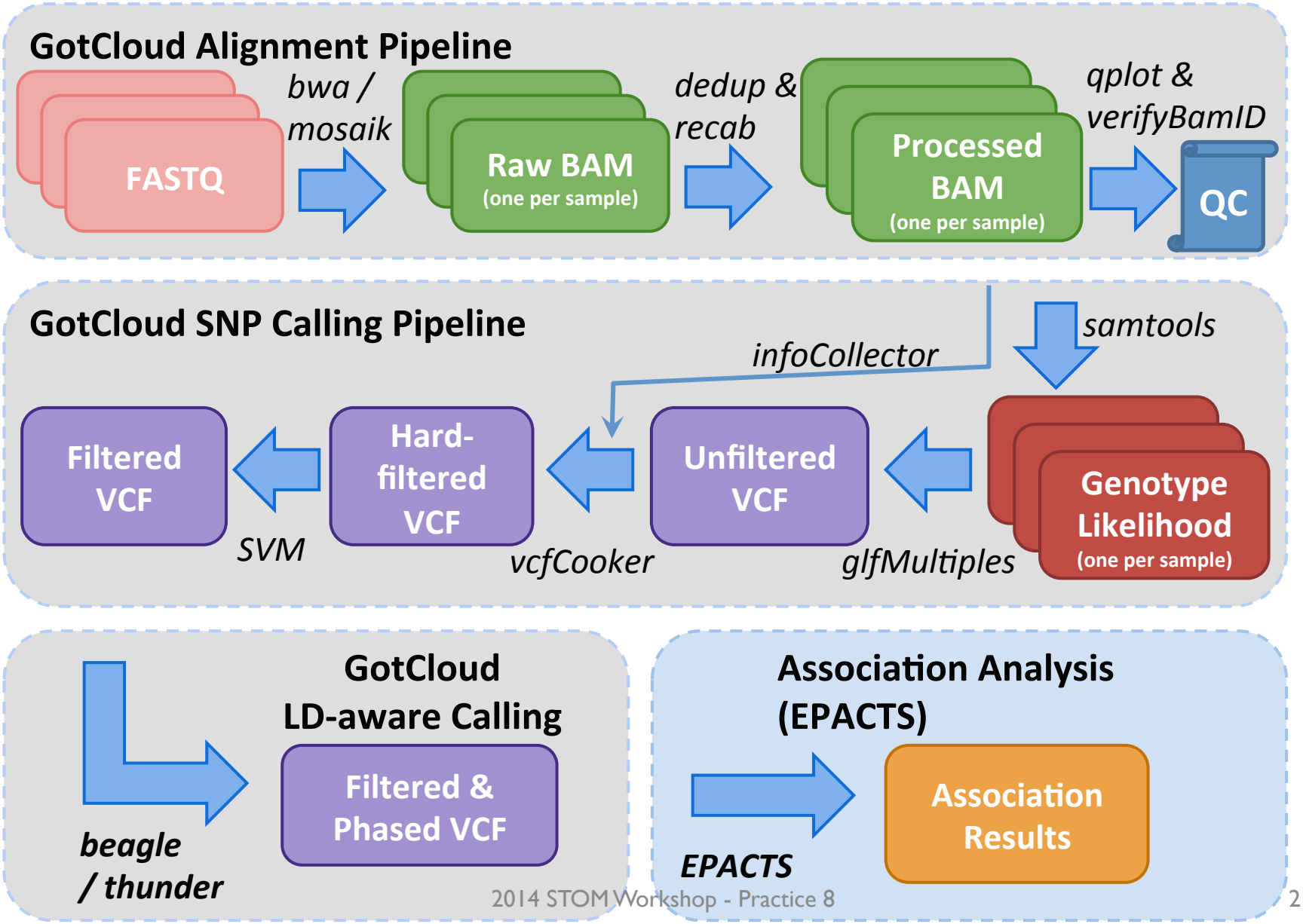
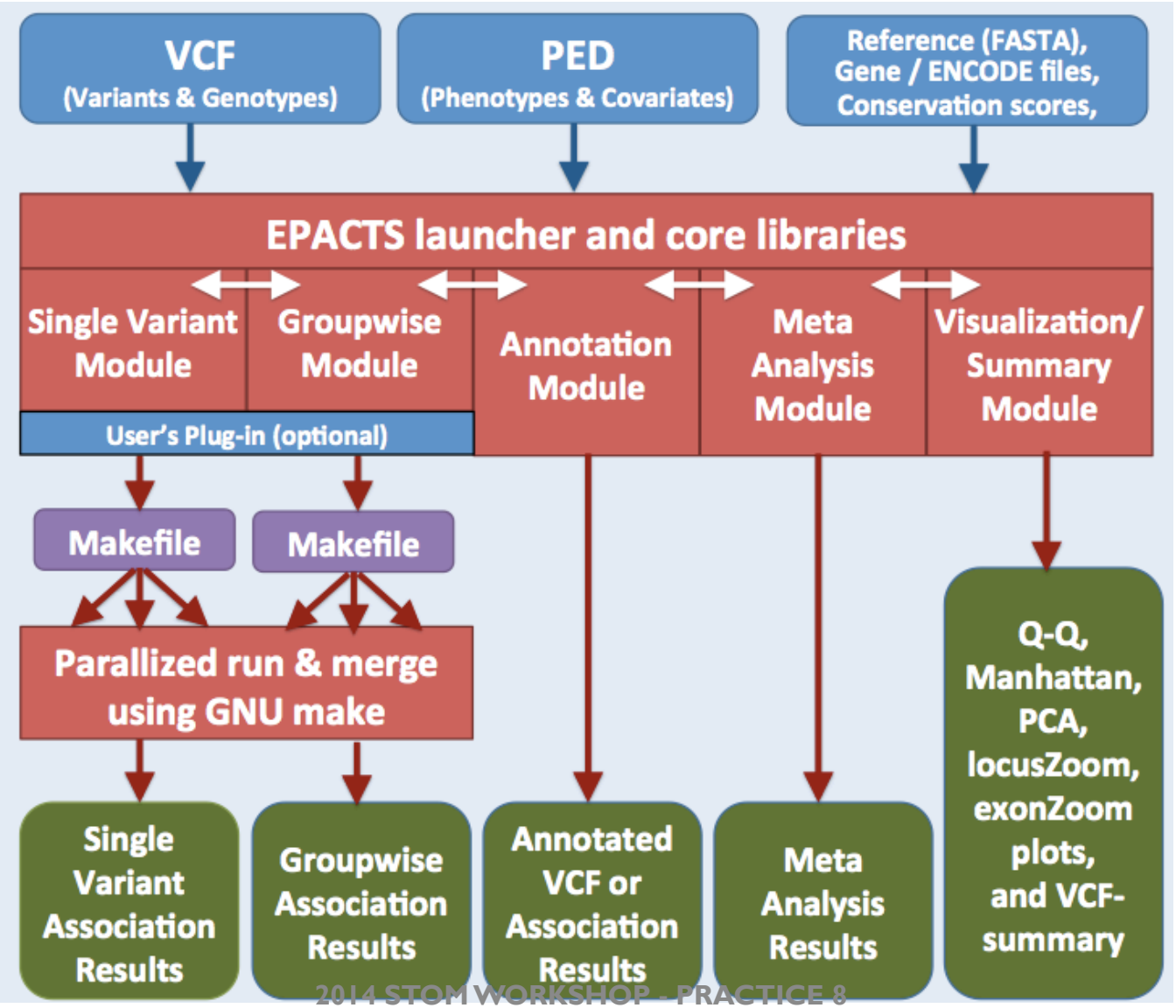
EPACTS Association Analysis Pipeline



OVERVIEW OF EPACTS FRAMEWORK



CHALLENGES IN SEQUENCE-BASED ASSOCIATION

- Much larger (10~100x) data size
  - Efficient and parallel computation is important

- Complex representation of variants and genotypes
  - SNPs, Indels, structural variations with multi-allelic variants
  - Genotypes with uncertainty across different depth and quality
  - Efficient implementation VCF (Variant Call Format) files is not simple

- Many methods are published, but only a few are usefully implemented.
  - Software implementation is becoming a major bottleneck
  - Need tools to transform “methods” to “software”

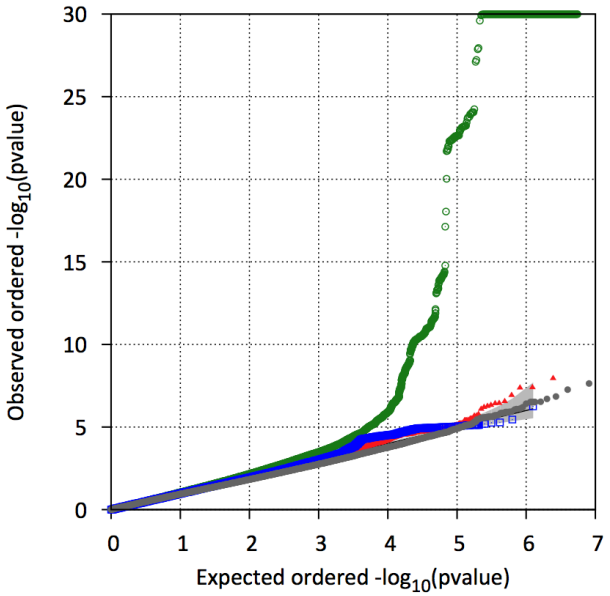
KEY FEATURES OF EPACTS

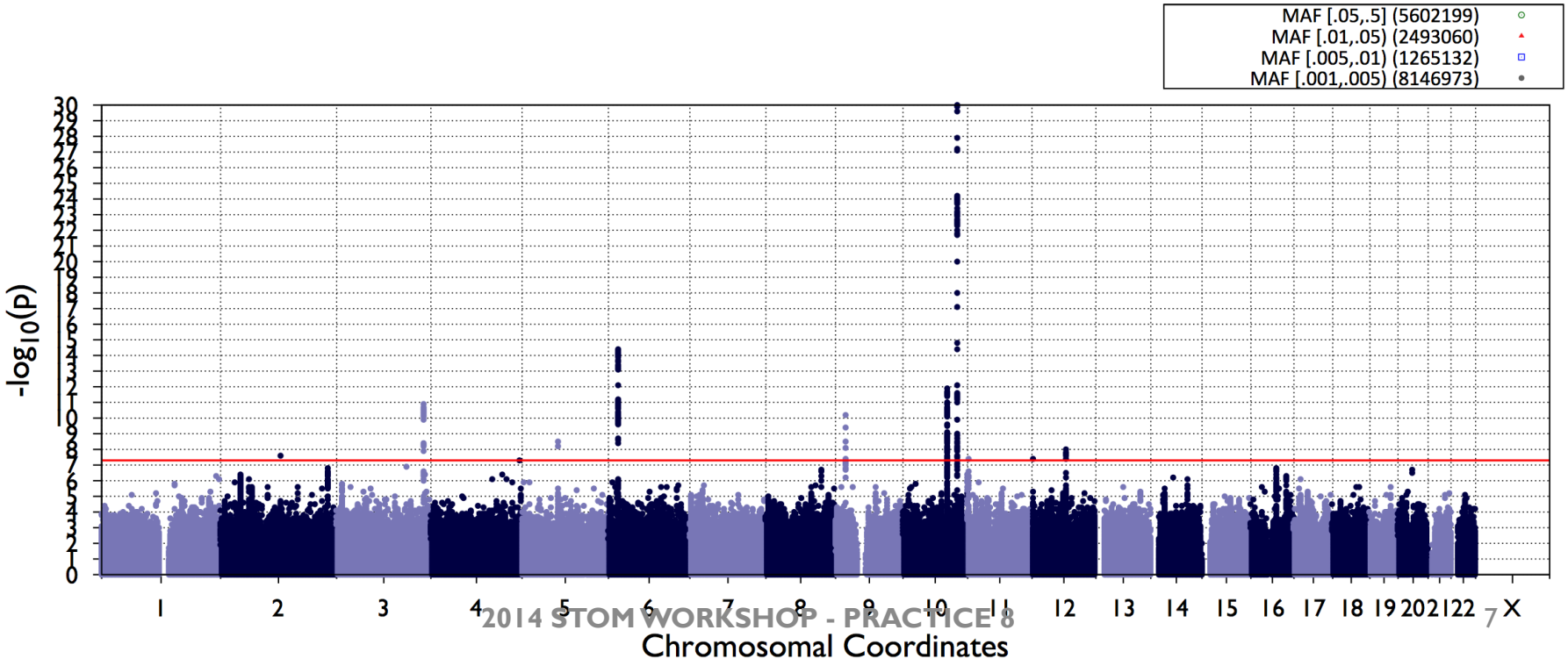
- Convenient and **dynamic plug-in** of user-defined statistical tests
  - Facilitate interaction between method developers and users
- **Efficient and parallel access of VCF** files
- **Fault-tolerant pipeline** structure based on GNU make
- Support of **a variety of single variant and groupwise tests**
- **Convenient** to run
  - All you need is just VCF and phenotype (PED) file
- **Automated visualization** of association signals and QC metrics
  - QQ-plot, Manhattan plot, PCA plot, LocusZoom plot
- Automated **annotation** of coding and noncoding variants
- Under **active development** more features are in progress (e.g. eQTL)

STATISTICAL TESTS AVAILABLE

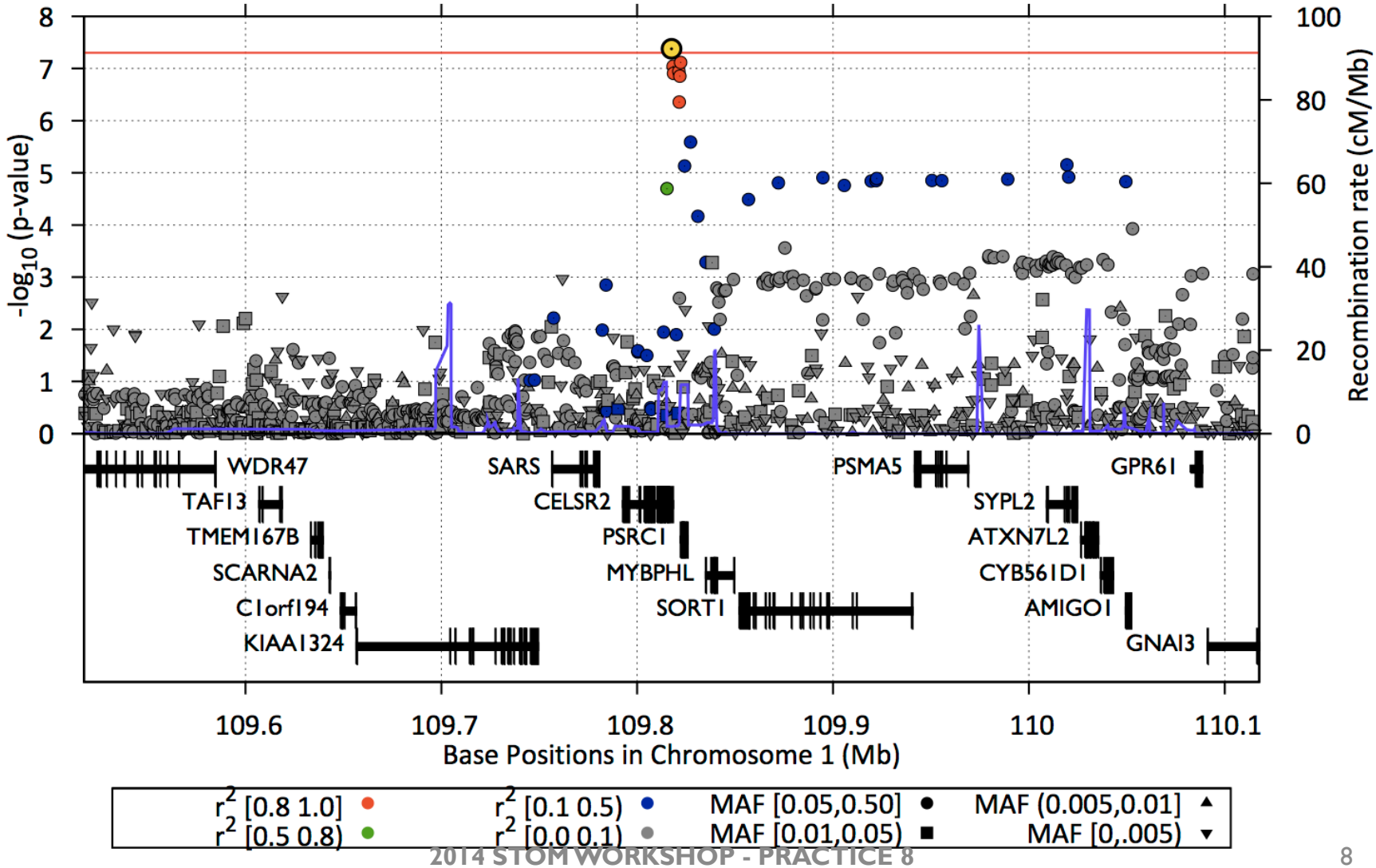
Single Variant Test	Groupwise Test
Wald Test	Collapsing
Score Test	Madsen-Browning*
Likelihood-ratio test	Reverse Regression
Firth bias-corrected LRT	SKAT / SKAT-O
Reverse Regression	VariableThreshold (VT)
Wilcoxon Rank Sum	EMMAX-Collapsing
EMMAX	EMMAX-VT

Example of Manhattan & QQ plots Automatically generated by EPACTS using a genome-wide data

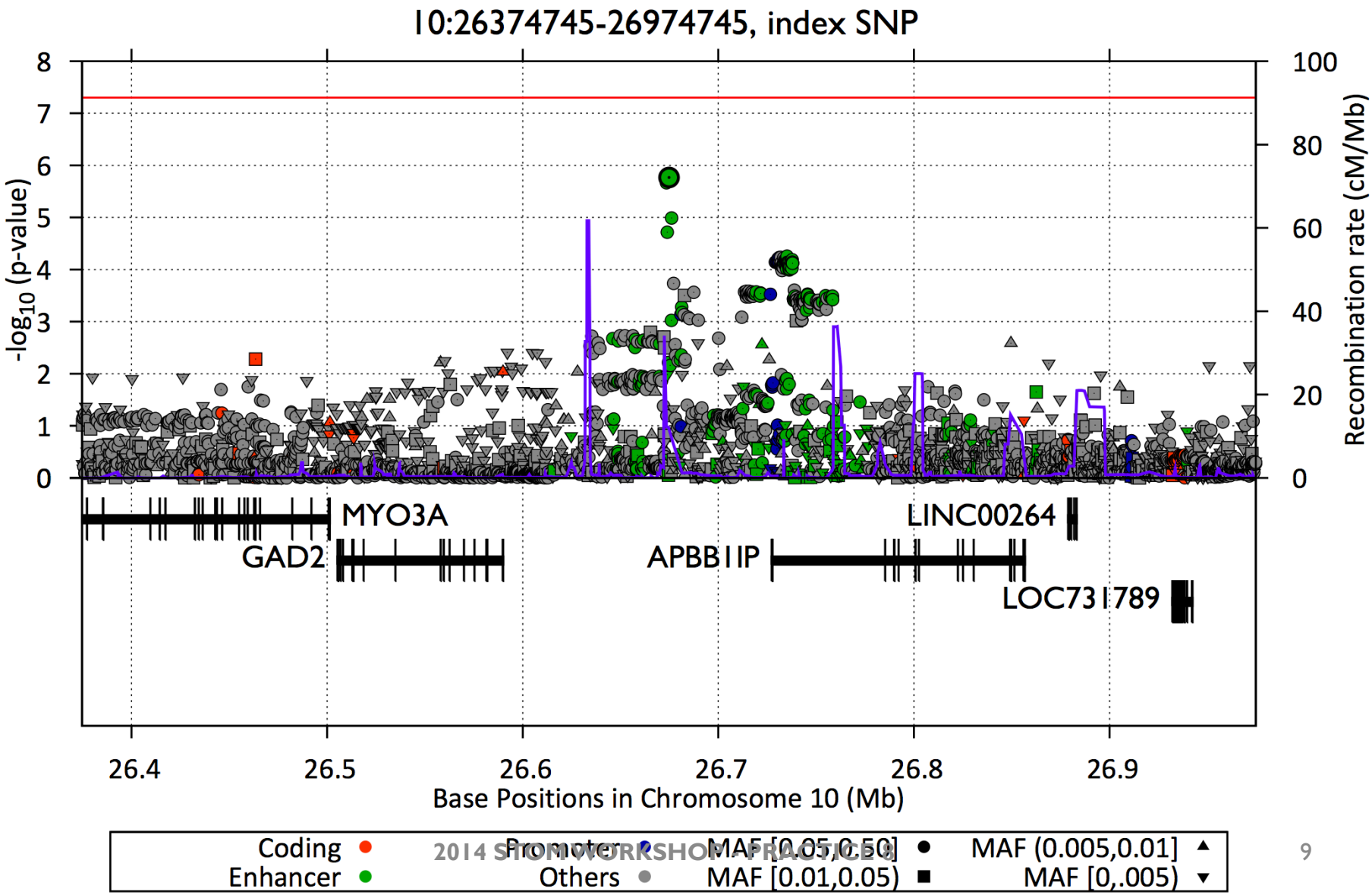




Zoom Plots for top associations



Zoom Plots by Regulatory Regions



GETTING STARTED WITH EPACTS

- Input Files – What should we provide?
  - VCF : genotype data (bgzipped and tabixed)
    - [prefix].vcf.gz and [prefix].vcf.gz.tbi should exist
  - PED : phenotype & covariate data
    - Header can be in a separate file (.dat) or in the first line (starting with #)
- Additional Input Files (Optional)
  - Marker group data (for groupwise test)
  - Reference genome sequence (for annotation)
  - Gene annotation files (in UCSC format)
  - ENCODE chromatin state predictions

SETTING ENVIRONMENTAL VARIABLES AGAIN

- Check if the files are still accessible

  ```
  ls /data/stom2014/session5/
  ```

  – If you see any errors, please let me know now!

- For convenience, let's set some variables. ~/out does not have to be created again

  ```
  export S5=/data/stom2014/session5
  ```

Example of Input PED File

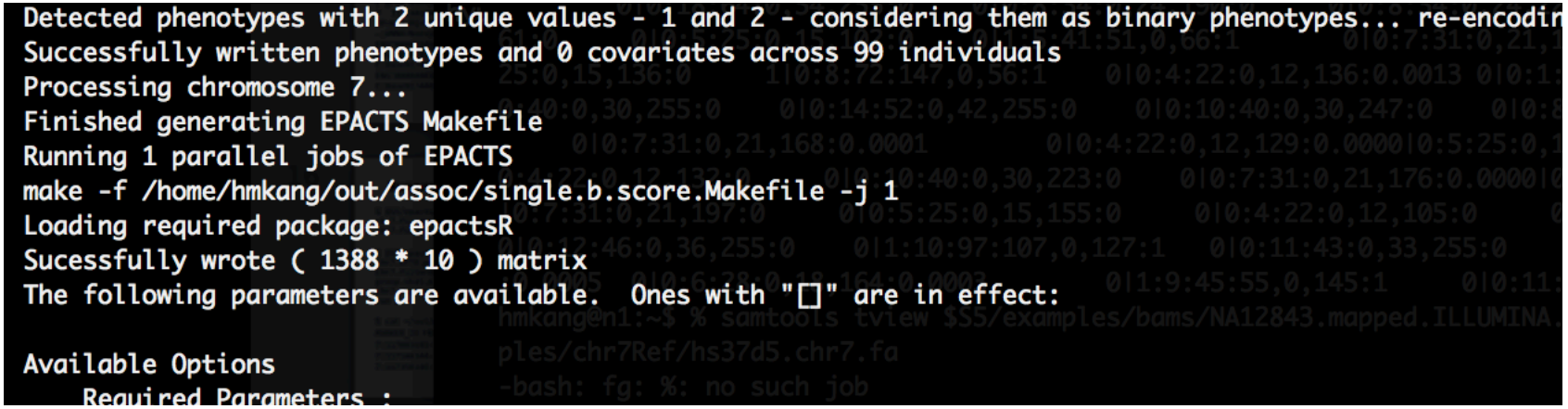
```
% less $S5/examples/index/chr7.CFTR.ped
```

#FAM_ID	IND_ID	DAD_ID	MOM_ID	SEX	PHENO	QT
NA06984	NA06984	0	0	0	1	-0.657149296617419
NA06985	NA06985	0	0	0	2	-0.449034856685281
NA06986	NA06986	0	0	0	1	0.0975849179986626
NA06989	NA06989	0	0	0	2	1.77095069670763
NA06994	NA06994	0	0	0	1	0.287475900193007
NA07000	NA07000	0	0	0	1	-1.36632872407691
NA07037	NA07037	0	0	0	2	-0.1278913321612
NA07048	NA07048	0	0	0	2	-1.45798868745693
NA07051	NA07051	0	0	0	1	-0.599618650565132

```
% ls $S5/out/snps/beagle/chr7/
chr7.filtered.PASS.beagled.vcf.gz
```

RUNNING SINGLE VARIANT ANALYSIS

```
% mkdir ~/out/assoc
% $S5/epacts/bin/epacts single --ped $S5/examples/index/
chr7.CFTR.ped --vcf $S5/out/snps/beagle/chr7/
chr7.filtered.PASS.beagled.vcf.gz --pheno PHENO --out ~/
out/assoc/single.b.score --test b.score --anno --ref $S5/
examples/chr7Ref/hs37d5.chr7.fa --region
7:117000000-117500000 --run 1
```



SINGLE VARIANT ASSOCIATION RESULTS

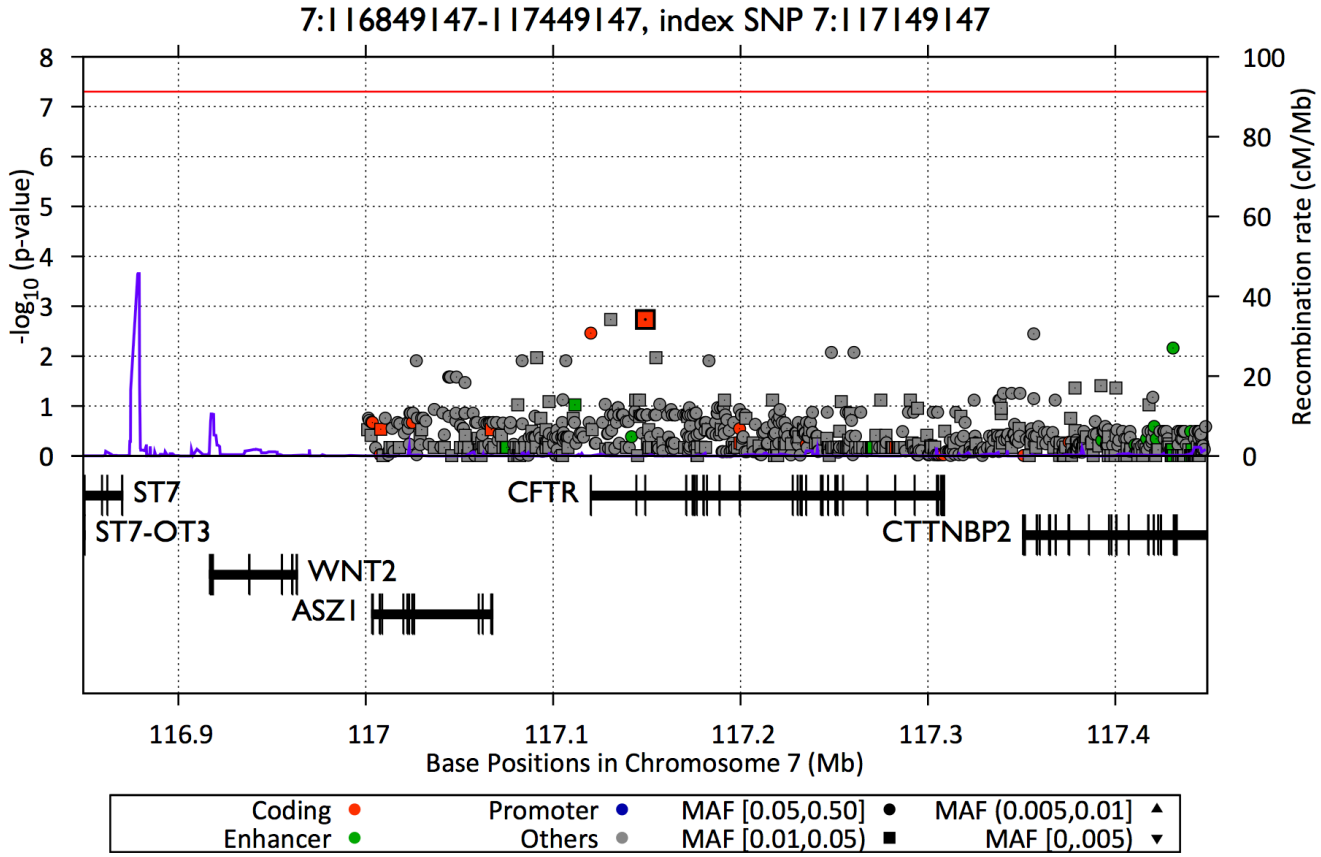
```
% head ~/out/assoc/single.b.score.epacts.top5000
```

#CHROM	BEGIN	END	MARKER_ID	NS	AC	CALLRATE	MAF	PVALUE
7	117130755		117130755	7:117130755_C/G_Intron:CFTR			99	9
7	117149147		117149147	7:117149147_G/A_Nonsynonymous:CFTR			99	
7	117120141		117120141	7:117120141_G/C_Utr5:CFTR			99	14
7	117356483		117356483	7:117356483_C/T_Intron:CTTNBP2			99	11
7	117459023		117459023	7:117459023_A/G_Intron:CTTNBP2			99	121
7	117430909		117430909	7:117430909_T/C_Intron:CTTNBP2			99	10
7	117456904		117456904	7:117456904_C/T_Intron:CTTNBP2			99	115
7	117457141		117457141	7:117457141_G/C_Intron:CTTNBP2			99	115
7	117467003		117467003	7:117467003_T/A_Intron:CTTNBP2			99	122

SCORE	NS.CASE	NS.CTRL	AF.CASE	AF.CTRL				
1	0.045455	0.0018407	3.1148	50	49	0.09	0	
9	1	0.045455	0.0018407	3.1148	50	49	0.09	0
1	0.070707	0.0034417	-2.9253	50	49	0.02	0.12245	
1	0.055556	0.0035695	-2.9139	50	49	0.01	0.10204	
1	0.38889	0.0047674	2.8223	50	49	0.71	0.5102	
1	0.050505	0.0068926	-2.702	50	49	0.01	0.091837	
1	0.41919	0.0076959	2.6651	50	49	0.68	0.47959	
1	0.41919	0.0076959	2.6651	50	49	0.68	0.47959	
1	0.38384	0.0077344	2.6634	50	49	0.71	0.52041	

Creating Zoom Plot

```
$S5/epacts/bin/epacts-zoom --vcf ~/out/snps/
chr7.filtered.PASS.beagled.anno.vcf.gz --pos
7:117149147 --prefix ~/out/assoc/single.b.score --
cellType Gm12878
```



VARIANT ANNOTATION WITH EPACTS

```
% $S5/epacts/bin/epacts anno --in ~/out/snps/beagle/chr7/
chr7.filtered.PASS.beagled.vcf.gz --out ~/out/snps/
chr7.filtered.PASS.beagled.anno.vcf.gz --ref $S5/
examples/chr7Ref/hs37d5.chr7.fa
```

```
% zcat ~/out/snps/chr7.filtered.PASS.beagled.anno.vcf.gz
| grep Nonsynonymous | grep CFTR | cut -f 1-8 | head -1
```

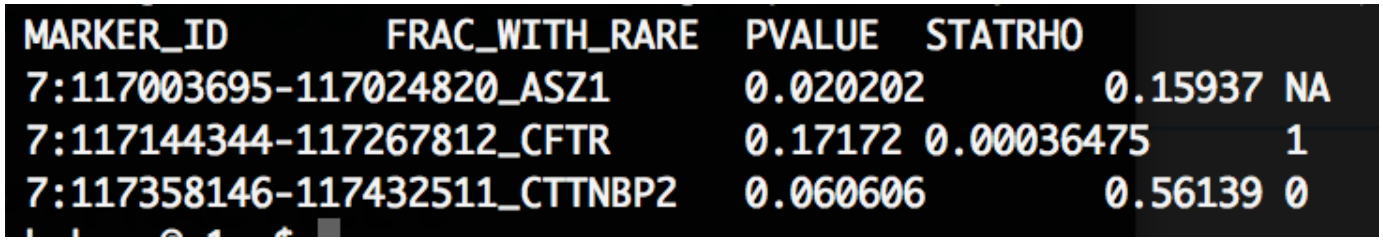
```
7       117144344       .       C       T       100     PASS    DP=689;MQ=58;NS=98;AN=198;AC=1;A
F=0.005323;AB=0.3278;AZ=-1.4260;FIC=-0.0056;SLRT=-0.0056;HWEAF=0.0053;HWDAF=0.0107,0.0000;LBS=0,
0,8,11,0,0,0,0;OBS=0,0,299,368,0,0,7,5;STR=-0.035;STZ=-0.921;CBR=-0.029;CBZ=-0.762;IOR=0.000;IOZ
=-1.018;AOI=-14.680;AOZ=-13.662;LQR=0.027;MQ0=0.000;MQ10=0.000;MQ20=0.000;MQ30=0.031;SVM=1.20661
;BAVGPOST=1.000;BRSQ=0.992;ANNO=Nonsynonymous:CFTR;ANNOFULL=CFTR/ENST00000546407.1:+:Exon:Noncod
ing|CFTR/ENST00000454343.1:+:Nonsynonymous(CGC/Arg/R->TGC/Cys/C:Base92/4260:Codon31/1420:Exon2/2
6):Exon|CFTR/ENST00000003084.6:+:Nonsynonymous(CGC/Arg/R->TGC/Cys/C:Base92/4443:Codon31/1481:Exo
n2/27):Exon
hmkang@n1:~$
```

RUNNING GENE-LEVEL GROUPWISE TEST

```
% $S5/epacts/bin/epacts make-group --vcf ~/out/snps/
chr7.filtered.PASS.beagled.anno.vcf.gz --out ~/out/snps/
chr7.filtered.PASS.beagled.anno.grp --nonsyn

% $S5/epacts/bin/epacts group --ped $S5/examples/index/
chr7.CFTR.ped --vcf ~/out/snps/
chr7.filtered.PASS.beagled.anno.vcf.gz --out ~/out/assoc/
group.skat.o --groupf ~/out/snps/
chr7.filtered.PASS.beagled.anno.grp --test skat --skat-o --
run 2

% cat ~/out/assoc/group.skat.o.epacts | cut -f 4,6,10,11
```



MARKER_ID	FRAC_WITH_RARE	PVALUE	STATRHO
7:117003695-117024820_ASZ1	0.020202	0.15937	NA
7:117144344-117267812_CFTR	0.17172	0.00036475	1
7:117358146-117432511_CTTNBP2	0.060606	0.56139	0

RUNNING GENE-LEVEL GROUPWISE TEST

```
% $S5/epacts/bin/epacts group --ped $S5/examples/
index/chr7.CFTR.ped --vcf ~/out/snps/
chr7.filtered.PASS.beagled.anno.vcf.gz --out ~/out/
assoc/group.VT --groupf ~/out/snps/
chr7.filtered.PASS.beagled.anno.grp --test VT --run 2
```

```
% cat ~/out/assoc/group.VT.epacts | cut -f 4,6,10,13
```

MARKER_ID	PASS_MARKERS	PVALUE	OPT_THRES_RAC
7:117003695-117024820_ASZ1	1	0.44	2
7:117144344-117267812_CFTR	7	2.7e-06	9
7:117358146-117432511_CTTNBP2	4	0.55	1

CREATING YOUR OWN TEST "mytest"

- For single variant test
  Create an R file : **`${EPACTS}/data/single.mytest.R`**

- For groupwise test
  Create an R file : **`${EPACTS}/data/group.mytest.R`**

- When running EPACTS association (single or groupwise), use **`--test mytest`** instead of other known test name

- Refer to existing code to learn the example
  `less $S5/epacts/share/EPACTS/single.q.lm.R`

SUMMARY : EPACTS

- Useful tool for association analysis for sequence data
- Widely used association tests are implemented
  - New tests can be easily plugged in
- Groupwise tests are also available
- Many automated tools available for easier use
  - Manhattan plot, QQ plot, zoom plot, annotation
- New features (partially available)
  - Functional enrichment analysis
  - eQTL analysis module
  - Meta-analysis

THANK YOU FOR LISTENING!