Revised problems, selected solutions and additional material: ISMB2011 tutorial: genetics of gene expression with R/bioc

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Contents

1 Selected problems from the main notes, with some extensions

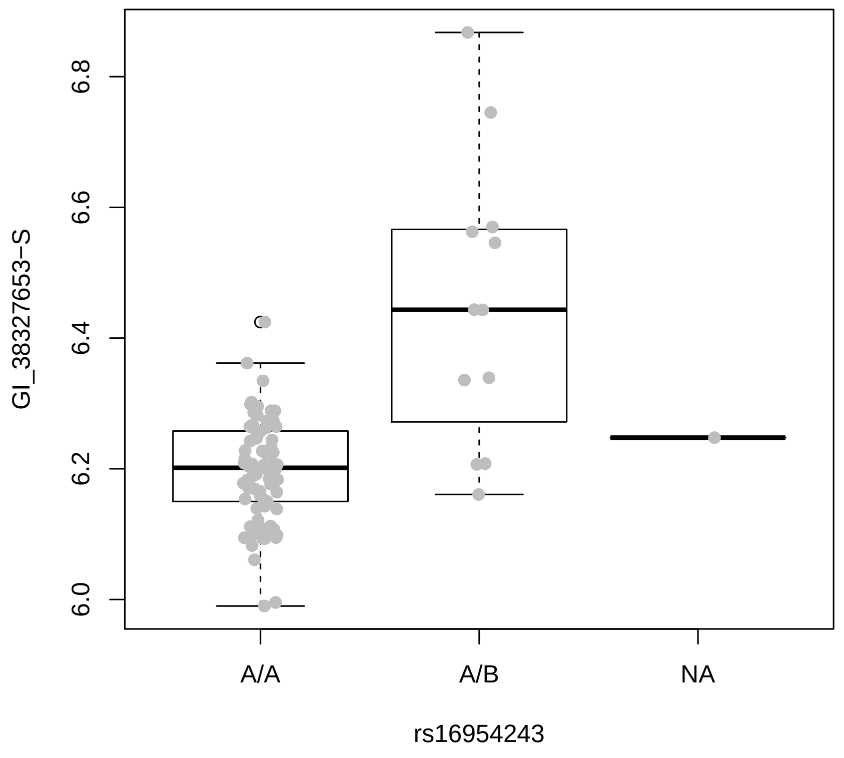
- **Manual computation of `gwSnpTests` results.** The basic result of interest is

```
> library(GGtools)
> library(ggtut)
> c17 = getSS("GGdata", "17", renameChrs = "chr17")
> t1 = gwSnpTests(genesym("CHRNE") ~ male, c17, chrnum("chr17"))
> topSnps(t1)
```

```
               p.val
rs16954243 2.926e-09
rs7214776  7.564e-09
rs8081611  7.564e-09
rs2302321  4.839e-08
rs8070572  2.506e-07
rs7225684  4.088e-07
rs2243093  8.157e-07
rs16954257 9.002e-07
rs2243100  9.274e-07
rs8077875  1.475e-06
```

The data configuration giving rise to the strongest association test is

```
> plot_EvG(probeId("GI_38327653-S"), rsid("rs16954243"), c17)
```



Of note is the sparsity of data on the A/B genotype.

To obtain the expression data serving as dependent variable,

```
> chrneExpr = as.numeric(exprs(c17[genesym("CHRNE"), ]))
```

The count of B alleles is obtained as

```
> num243 = as(smList(c17)[["chr17"]][, "rs16954243"], "numeric")
```

A simple approach to testing the additive genetic model with adjustment for gender is

```
> lm1 = lm(chrneExpr ~ num243 + male, data = pData(c17))
> summary(lm1)

Call:
lm(formula = chrneExpr ~ num243 + male, data = pData(c17))

Residuals:
     Min       1Q   Median       3Q      Max
```

```
-0.2805 -0.0483 -0.0019  0.0606  0.4042

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   6.1868     0.0164  377.55  < 2e-16 ***
num243        0.2545     0.0333    7.65  2.7e-11 ***
maleTRUE      0.0221     0.0227    0.97     0.33
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.107 on 86 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared: 0.41,       Adjusted R-squared: 0.396
F-statistic: 29.8 on 2 and 86 DF,  p-value: 1.43e-10
```

The computations in `gwSnpTests` occur via `snp.rhs.tests` in the `snpMatrix` package:

```
> c17$chrneExpr = chrneExpr
> lm2 = snp.rhs.tests(chrneExpr~male,
+  snp.data=smList(c17)[[1]], data= pData(c17),
+  family="gaussian")
> lm2["rs16954243",]

           Chi.squared Df   p.value
rs16954243       35.23  1 2.926e-09
```

Comments:

- The least-squares based p-value and the `snp.rhs.tests` p-values differ as the latter is based on the score test of the additive genetic effect after adjustment for covariates.
- We have disregarded familial structure in the CEU data. One approach to coping with this involves a cluster-adjusted robust variance estimate in the score test, but in relatively small samples this seems to result in severe loss of power.

  ```
  > table(table(c17$famid))

   3  6
  10 10
  ```

  ```
  > lm3 = snp.rhs.tests(chrneExpr~male+cluster(famid),
  +  snp.data=smList(c17)[[1]], data=
  +  pData(c17), family="gaussian")
  > lm3["rs16954243",]
  ```

```
           Chi.squared Df  p.value
rs16954243       7.841  1 0.005107
```

If instead we allow a random effect of family, we have

```
> library(nlme)
> lme1 = lme(chrneExpr~num243+male, random=~1|famid,
+   data=pData(c17), na.action=na.omit)
> summary(lme1)
```

```
Linear mixed-effects model fit by REML
 Data: pData(c17)
     AIC    BIC logLik
  -120.8 -108.5  65.39

Random effects:
 Formula: ~1 | famid
        (Intercept) Residual
StdDev:     0.02655   0.1039

Fixed effects: chrneExpr ~ num243 + male
            Value Std.Error DF t-value p-value
(Intercept) 6.186   0.01705 67   362.7  0.0000
num243      0.259   0.03281 67     7.9  0.0000
maleTRUE    0.023   0.02211 67     1.0  0.2978
 Correlation:
         (Intr) num243
num243   -0.247
maleTRUE -0.621 -0.014

Standardized Within-Group Residuals:
     Min       Q1      Med       Q3      Max
-2.39632 -0.58167  0.05751  0.57956  3.67920

Number of Observations: 89
Number of Groups: 20
```

Other things being equal, the best way for dealing with familial correlation in this setting is to use an appropriate variance components model like that afforded by `lme`. We do not address this issue further, and have ignored the correlation to illustrate mechanics of analysis.

– Counting SNP that are monomorphic in the cohort.

```
> cs17 = col.summary(smList(c17)[[1]])
> dim(cs17)
```

```
[1] 89701     9
```

```
> cs17[1:5, ]
```

```
           Calls Call.rate Certain.calls    RAF     MAF    P.AA    P.AB    P.B
rs6565733     90         1             1 0.8722 0.12778 0.01111 0.23333 0.7555
rs1106175     90         1             1 0.1833 0.18333 0.67778 0.27778 0.0444
rs17054921    90         1             1 0.9889 0.01111 0.00000 0.02222 0.9777
rs8064924     90         1             1 0.8778 0.12222 0.00000 0.24444 0.7555
rs8070440     90         1             1 0.9444 0.05556 0.00000 0.11111 0.8888
             z.HWE
rs6565733   0.4440
rs1106175  -0.6864
rs17054921  0.1066
rs8064924   1.3210
rs8070440   0.5580
```

```
> sum(cs17[, "MAF"] == 0)
```

```
[1] 28734
```

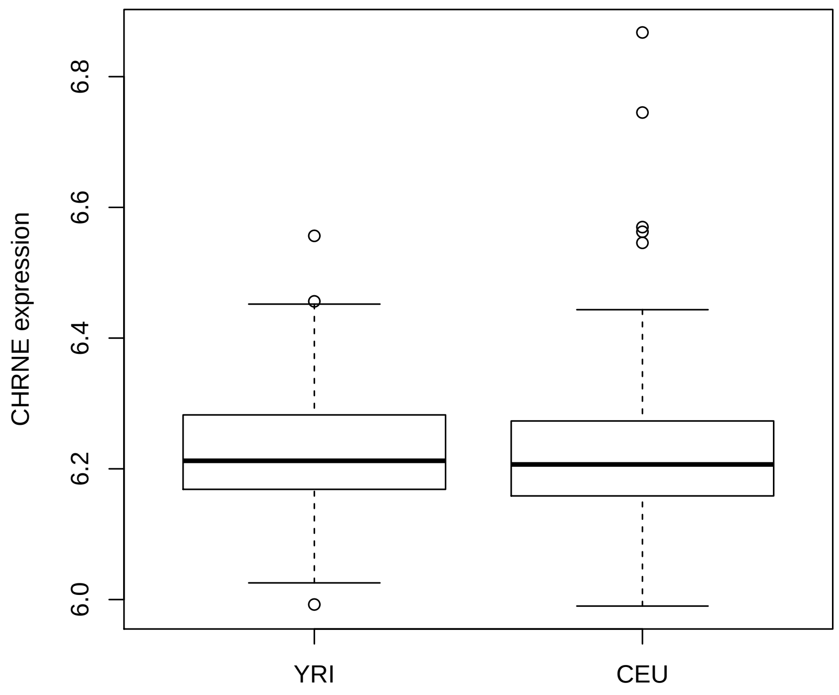
– Checking a finding in another population; sensitivity analysis. We supply the analogous expression and genotype information on the Yoruba cohort in the `hmyriB36` package.

```
> y17 = getSS("hmyriB36", "17", renameChrs = "chr17")
> y1 = gwSnpTests(genesym("CHRNE") ~ male, y17, chrnum("chr17"))
> topSnps(y1)
```

```
              p.val
rs9889685  0.0001236
rs7212518  0.0001347
rs9907560  0.0001407
rs6501801  0.0001414
rs2024498  0.0001432
rs8069187  0.0001432
rs9891980  0.0001432
rs9303366  0.0001432
rs1917997  0.0001461
rs16974160 0.0001681
```

We check to see if the marginal distributions of expression in the two populations are similar:

```
> ych = exprs(y17[genesym("CHRNE"), ])
> cch = exprs(c17[genesym("CHRNE"), ])
> boxplot(list(YRI = ych, CEU = cch), ylab = "CHRNE expression")
```



These are reasonably similar distributions – there is a little more dynamic range for this gene in CEU. So we can't readily argue that the expression measures in YRI are somehow compromised for this gene, depressing power. A useful exercise would be to assess the expression measures in CEU for outlying values and check the sensitivity of the eQTL inference to these values. Winsorization might be considered.

```
> library(parody)
> calout.detect(chrneExpr)
```

```
$ind
[1] 63  7 52 44 49

$val
[1] 6.868 6.745 6.570 6.563 6.546
```

This procedure uses a Gaussian reference model and outward testing to objectively reason about the existence of outlying observations. We can repeat our manual test of association simply excluding the outliers

```
> summary(update(lm1, subset = chrneExpr < 6.5))
```

```
Call:
lm(formula = chrneExpr ~ num243 + male, data = pData(c17), subset = chrneExpr
    6.5)

Residuals:
     Min       1Q   Median       3Q      Max 
-0.21084 -0.05519  0.00614  0.06327  0.22366 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   6.2009     0.0129  482.63   <2e-16 ***
num243        0.1064     0.0328    3.24   0.0017 ** 
maleTRUE     -0.0073     0.0182   -0.40   0.6892    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 

Residual standard error: 0.0826 on 81 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared: 0.12,	Adjusted R-squared: 0.0987 
F-statistic: 5.55 on 2 and 81 DF,  p-value: 0.00553 
```

This shows the CEU result to be quite sensitive to the extreme expression values achieved for the heterozygous individuals. Another sensitivity analysis is Winsorization (see section 3.2.1 of V. Barnett and T. Lewis, *Outliers in statistical data*, for a discussion of Winsorization for mean estimation; Winsorization of covariates is not widely studied.)

```
> winch = chrneExpr
> winch[winch >= 6.5] = 6.5
> summary(update(lm1, winch ~ .))
```

```
Call:
lm(formula = winch ~ num243 + male, data = pData(c17))

Residuals:
     Min       1Q   Median       3Q      Max 
-0.22250 -0.05158  0.00559  0.06645  0.23023 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)  6.19435    0.01344  461.04  < 2e-16 ***
num243       0.18888    0.02727    6.93  7.4e-10 ***
maleTRUE     0.00636    0.01864    0.34     0.73    
---
```

```
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.0879 on 86 degrees of freedom
  (1 observation deleted due to missingness)
Multiple R-squared: 0.359,       Adjusted R-squared: 0.344
F-statistic: 24.1 on 2 and 86 DF,  p-value: 4.98e-09
```

The lesson is that sensitivity analysis by excluding potentially aberrant values can be very costly; softer approaches are feasible, and in this case suggest that sensitivity of the eQTL finding to achieved high values of CHRNE expression in CEU may not be so great.

For now we conclude that data from the YRI cohort seem provide no evidence of an eQTL for CHRNE, in contrast to the CEU cohort.

– **Approximate FDR for distance-delimited tests (original notes exercise, p. 18).**

We saved the results of `cisProxScores` applied to the surveys before and after permutation. We can obtain the collections of delimited scores as follows:

```
> data(CPS17)
> data(PERMCPS17)
> sb1 = scoresByGenes(CPS17, as.GRanges = FALSE)
> permsb1 = scoresByGenes(PERMCPS17, as.GRanges = FALSE)
> summary(sapply(sb1, length))
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
    1.0    52.0    71.0    86.3    96.0   696.0
```

Let's focus on the strongest association observed per gene within 50kb of its coding region. The permutation distribution has quantiles

```
> targp = c(0.95, 0.975, 0.99, 0.995)
> pq = quantile(sapply(permsb1, max), targp)
> pq
```

```
   95%  97.5%    99%  99.5% 
 9.951 11.279 12.886 13.565 
```

leading to counts of false eQTL claims

```
> fp = sapply(pq, function(x) sum(sapply(permsb1, max) > x))
> fp
```

```
  95% 97.5%   99% 99.5% 
   24    12     5     3 
```

The numbers of real tests passing these thresholds are

```
> op = sapply(pq, function(x) sum(sapply(sb1, max) > x))
> op
```

```
  95% 97.5%   99% 99.5%
  113    88    70    62
```

so a table of thresholds and approximate FDRs is

```
> fdrmat = cbind(pctile.null = targp * 100, thresh = pq, nfalse = fp,
+     nsig = op, fdr = fp/op)
> fdrmat
```

```
      pctile.null thresh nfalse nsig     fdr
95%          95.0  9.951     24  113 0.21239
97.5%        97.5 11.279     12   88 0.13636
99%          99.0 12.886      5   70 0.07143
99.5%        99.5 13.565      3   62 0.04839
```

So out of our 498 genes on chromosome 17, we assert existence of 62 eQTL within 50kb at an FDR of about 5%. Let's look at the data configuration for the statistically weakest claim.

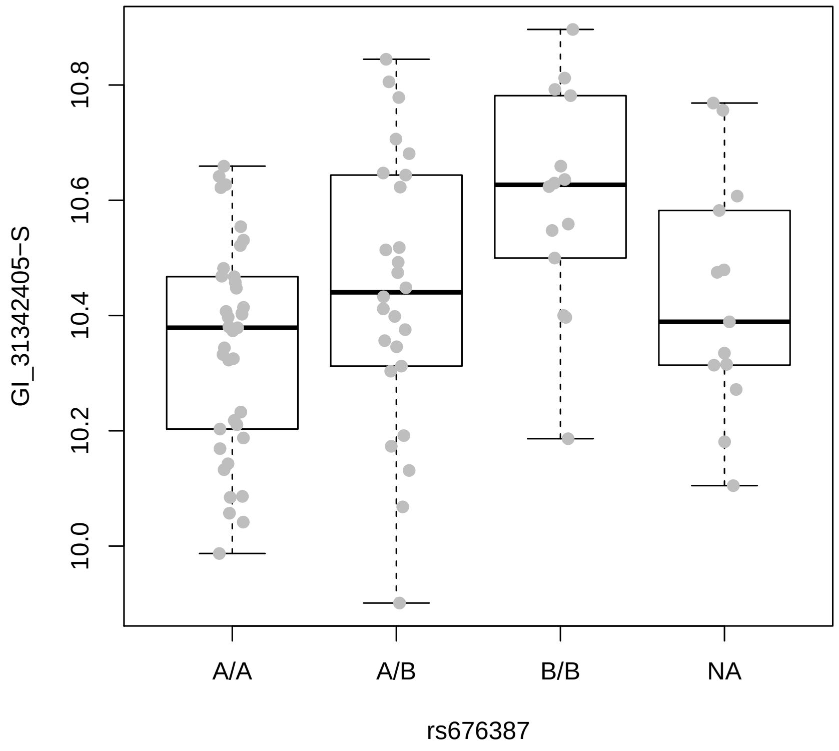
```
> at5p = which(sapply(sb1,max)>13.565) # find genes with
>                                      # sufficient scores
> sort(sapply(sb1[at5p],max))[1:5] # look at the weakest
```

```
GI_31342405-S  GI_4557308-S GI_21687219-S  GI_4759187-S GI_41327725-S
        13.59         13.83         13.90         13.99         14.25
```

```
>                                   # find strongest SNP
> sort(sb1[["GI_31342405-S"]], decreasing=TRUE)[1:5]
```

```
  rs676387 rs10454087     rs6963   rs646123   rs665268
     13.59      12.97      12.76      12.18      12.18
```

```
> plot_EvG(probeId("GI_31342405-S"), rsid("rs676387"), c17)
```



Not bad. Does it hold up in YRI?

– **Distinguishing variants in exons (continuation of problem on p. 18 of original notes).** The task here is to acquire the locations of the SNPs of interest. We'll focus on the highest-scoring SNP for each gene, restricting attention to associations with FDR $< 5\%$.

Retrieve the names of these most associated SNPs:

```
> snpsat5 = sapply(lapply(sb1[at5p],sort,decreasing=TRUE),
+       function(x)names(x[1]))
```

Get their locations:

```
> data(snpgr17)
> snpgr17[1:3]
```

```
GRanges with 3 ranges and 0 elementMetadata values
          seqnames       ranges strand |
             <Rle>    <IRanges>  <Rle> |
rs1106176    chr17 [6934, 6934]      * |
rs6420494    chr17 [7214, 7214]      * |
rs6420495    chr17 [7242, 7242]      * |
seqlengths
 chr17
    NA
```

Now find the proportion of these SNPs that are in exons:

```
> tx17 = hg18tx()  # get GenomicFeatures resource
> ex17 = exons(tx17, vals=list(exon_chrom="chr17"))
> mean(snpgr17[snpsat5] %in% ex17)
```

```
[1] 0.2903
```

The not-yet-released VariantAnnotation package can provide more information. We need to provide allele details for our variants, which we have not recorded or retained. However, since these are documented SNP, we have access to the reference assignments.

We will use the hg18-associated dbSNP data:

```
> library(SNPlocs.Hsapiens.dbSNP.20090506)
> sl17 = getSNPlocs("chr17")
```

We need to acquire the IUPAC codes for the SNPs of interest.

```
> AT5 = snpgr17[snpsat5]
> Nat5 = names(AT5)
> Nat5f = gsub("rs", "", Nat5)
> sig17 = sl17[match(Nat5f, sl17[, 1]), ]
> icodes = IUPAC_CODE_MAP[sig17[, "alleles_as_ambig"]]
> icodes[1:5]
```

```
   S    Y    M    K    R
"CG" "CT" "AC" "GT" "AG"
```

We will arbitrarily assign these as ref and variant for input to the predictCoding function of VariantAnnotation:

```
> c1 = sapply(strsplit(icodes, ""), "[", 1)
> c2 = sapply(strsplit(icodes, ""), "[", 2)
> elementMetadata(AT5)$ref = c1
> elementMetadata(AT5)$var = c2
```

We need the hg18 sequence:

```
> library(BSgenome.Hsapiens.UCSC.hg18)
```

Finally, we obtain the transcript database and call the predictCoding function to see which of the significant eQTL correspond to nonsynonymous modifications.

```
> library(VariantAnnotation)
> predictCoding(AT5, tx17, Hsapiens, "ref", "var")
```

```
DataFrame with 7 rows and 10 columns
  queryIndex        txID       refCodon       varCodon         refAA
   <integer> <character> <DNAStringSet> <DNAStringSet> <AAStringSet>
1         22       52441            ACT            ACT             T
2         27       54786            CAG            GAG             Q
3         27       54787            CAG            GAG             Q
4         27       54788            CAG            GAG             Q
5         44       55846            CGG            CGG             R
6         49       52805            GTA            GGA             V
7         49       52807            GTA            GGA             V
          varAA   Consequence    cds_id    cds_name exon_rank
  <AAStringSet>   <character> <integer> <character> <integer>
1             T    synonymous    176944          NA         2
2             E nonsynonymous    176945          NA         3
3             E nonsynonymous    176946          NA         4
4             E nonsynonymous    176947          NA         5
5             R    synonymous    176948          NA         6
6             G nonsynonymous    176949          NA         7
7             G nonsynonymous    176950          NA         8
```

– **Systematic assessment of effect of reducing expression heterogeneity via PCA (p.28)**

  We used gwSnpTests to illustrate, for one gene, the impact of employing principal components as 'adjustments' to eQTL test procedures. The motivations are detailed in papers by O. Stegle, Jeffrey Leek et al. Here we show how to introduce this approach in a chromosome-wide survey.

  We obtain the expression and genotype data, compute PCs on the expression matrix, and then reduce the expression set to the filtered 498 genes.

```
> c17 = getSS("GGdata", "17", renameChrs="chr17")
```

```
uncaching chr17
```

```
> pct = prcomp(t(exprs(c17)))
> o17 = observed17ceu()
> pm = probesManaged(o17,1) # names of filtered probes
> c17f = c17[probeId(pm),]  # subset
```

  We add the first four PC as 'phenoData' to the smlSet instance.

```
> c17f$PC1 = pct$x[, 1]
> c17f$PC2 = pct$x[, 2]
> c17f$PC3 = pct$x[, 3]
> c17f$PC4 = pct$x[, 4]
```

  On a 2-core MacBook Pro, the following takes only a few moments.

```
> library(multicore)
> if (!exists("eqtpc")) eqtpc = eqtlTests(c17f, ~male + PC1 + PC2 +
+     PC3 + PC4, targdir = "pc4", shortfac = 10, genegran = 2,
+     geneApply = mclapply)
```

Obtain the vector of best per-gene eQTL scores:

```
> adjtop = mclapply(pm,
+    function(x)topFeats(probeId(x), mgr=eqtpc, ffind=1))
> names(adjtop) = pm
> adjmax = sapply(adjtop, max)
```

Repeat the process for one permutation of expression against genotype:

```
> set.seed(1234);
> if (!exists("eqtpcPERM"))
+ eqtpcPERM = eqtlTests(permEx(c17f),
+    ~male+PC1+PC2+PC3+PC4, targdir="pc4_perm",
+    shortfac=10, genegran=2, geneApply=mclapply)
> adjtop_PERM = mclapply(pm,
+    function(x)topFeats(probeId(x), mgr=eqtpcPERM, ffind=1))
> names(adjtop_PERM) = pm
> adjmax_PERM = sapply(adjtop_PERM, max)
```

Now we tabulate the significance claims:

```
> pcts = c(0.95, 0.975, 0.99, 0.995)
> permq = quantile(adjmax_PERM, pcts)
> nfalse = sapply(permq, function(x) sum(adjmax_PERM > x))
> nsig = sapply(permq, function(x) sum(adjmax > x))
> options(digits = 3)
> cbind(PPCT = 100 * pcts, thresh = permq, nfalse = nfalse, nsig = nsig,
+     fdr = nfalse/nsig)

      PPCT thresh nfalse nsig    fdr
95%   95.0   24.3     25   82 0.3049
97.5% 97.5   27.3     13   60 0.2167
99%   99.0   29.9      4   47 0.0851
99.5% 99.5   32.2      3   39 0.0769
```

Compare to the unadjusted analysis:

```
> o17 = observed17ceu()
> p17 = onePerm17ceu()
> unadjtop_PERM = mclapply(pm, function(x) topFeats(probeId(x),
+     mgr = p17, ffind = 1))
> unadjmax_PERM = sapply(unadjtop_PERM, max)
> unadjtop = mclapply(pm, function(x) topFeats(probeId(x), mgr = o17,
+     ffind = 1))
> unadjmax = sapply(unadjtop, max)
> pcts = c(0.95, 0.975, 0.99, 0.995)
> permq = quantile(unadjmax_PERM, pcts)
> nfalse = sapply(permq, function(x) sum(unadjmax_PERM > x))
> nsig = sapply(permq, function(x) sum(unadjmax > x))
> options(digits = 3)
> cbind(PPCT = 100 * pcts, thresh = permq, nfalse = nfalse, nsig = nsig,
+     fdr = nfalse/nsig)
```

```
      PPCT thresh nfalse nsig   fdr
95%   95.0   23.5     25   45 0.556
97.5% 97.5   26.3     13   30 0.433
99%   99.0   30.7      5   22 0.227
99.5% 99.5   32.2      3   20 0.150
```

We see that this very simple adjustment may substantially affect power of this approach to eQTL detection. Application of SVA for a systematic survey seems more laborious. The PEER method of Stegle et al. should also be investigated.

– **Working with trans tests.**

On p. 33 the exercise is: Find a locus giving a $\chi^2(1)$ statistic exceeding 11.8 for association with FDPS; for this locus FDPS is the fourth-largest association score. Display the expression-vs- genotype plot. How would you perform a more comprehensive check for FDPS without restricting to MAF > 10%?

We are given

```
> tr17 = tr17_1_9()
> tr17
```

```
transManager instance, created Wed May 11 16:22:34 2011
dimension of scores component:
 number of loci checked: 44638; genes retained: 20
the call was:
transScores(smpack = "GGdata", snpchr = "17", rhs = ~male, targdirpref = "twfi
    geneApply = mclapply, chrnames = c("chr1", "chr9"), wrapperEndo = function
        low = 0.1))
```

```
> getClass(class(tr17))
```

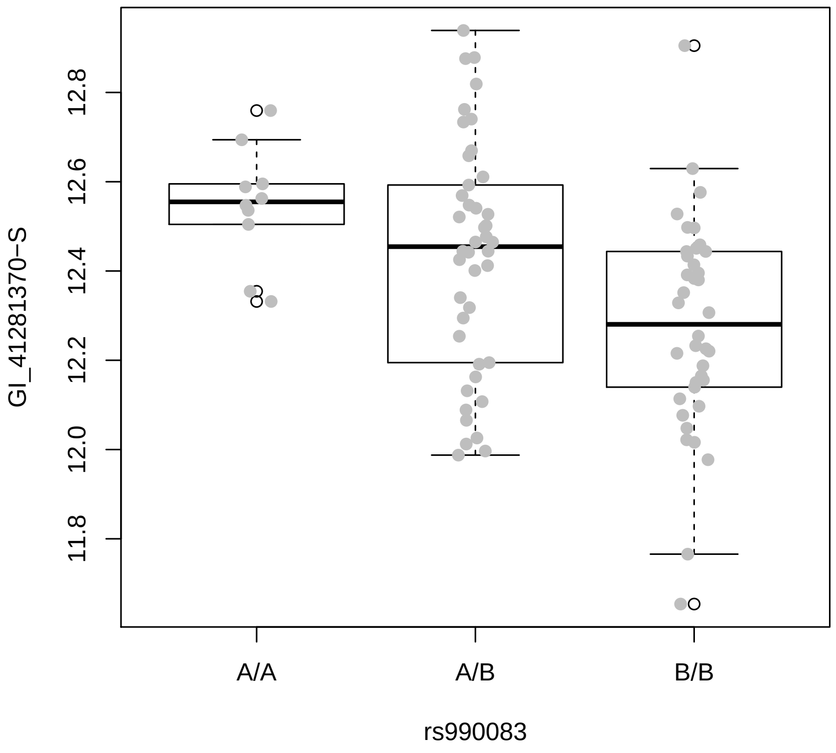
```
Class "transManager" [package "GGtools"]

Slots:

Name:  base
Class: list
```

```
> names(tr17@base)

[1] "scores"   "inds"     "guniv"    "snpnames" "call"     "date"     "shortfa
```

The exercise concerns working effectively with this fairly complicated data structure. The `scores` element has rows corresponding to SNP on chr17, and columns corresponding to ordered eQTL scores. The `i,j` element of `scores` is the jth highest score achieved for locus i. The `inds` element maps from scores to genes, through the `guniv` element. `guniv[ inds[i, j] ]` is the identifier for the gene giving rise to the jth highest score for locus i, or, more precisely the locus named by `snpnames[i]`.

To solve the problem:

```
> fdid = get("FDPS", revmap(illuminaHumanv1SYMBOL))
> fdind = which(tr17@base$guniv == fdid)
> posslocs = which(tr17@base$inds[, 4] == fdind)
> possloc = tr17@base$snpnames[33649]
> plot_EvG(probeId("GI_41281370-S"), rsid(possloc), c17)
```



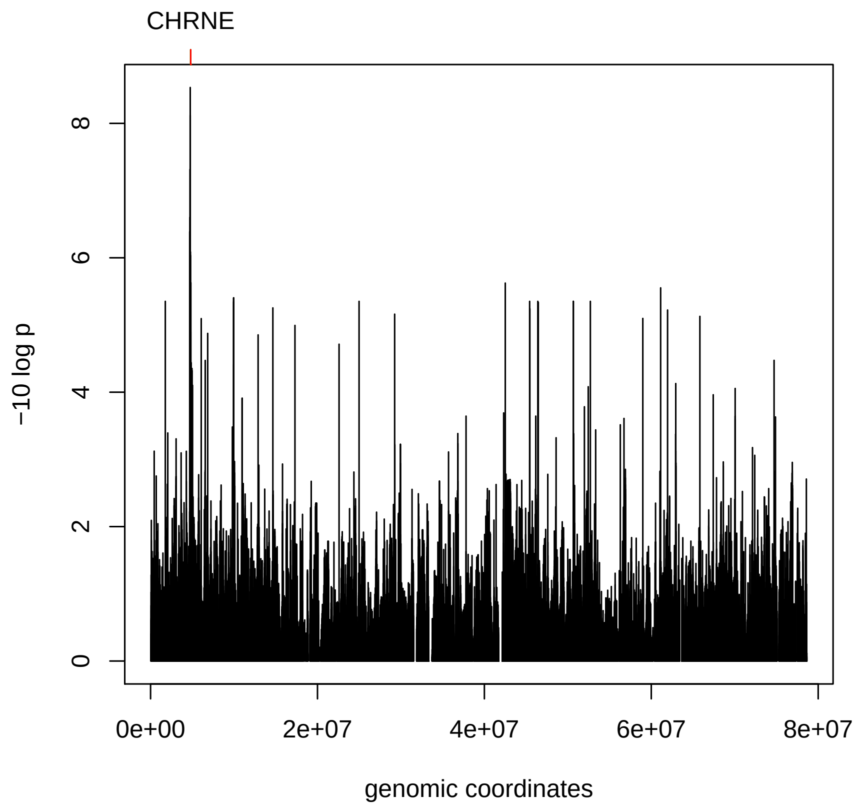
- **Visualizing an eQTL landscape in R**

In the main notes we have emphasized passage of test results to the genome browser as this approach delivers better pan and zoom capabilities in a metadata-rich context. However, it can be useful to create visualizations in R, as they are readily customized.

```
> mplot = function (css, sloc, ...)
+ {
+     pv = p.value(css@.Data[[1]])
+     sn = css@.Data[[1]]@snp.names
+     names(pv) = sn
+     slnames = names(sloc)
+     pv = -log10(pv[intersect(slnames, names(pv))])
+     sloc = sloc[names(pv)]
+     plot(start(sloc), pv, xlab = "genomic coordinates",
+         ylab = "-10 log p", pch="", ...)
+     segments(start(sloc), rep(0,length(pv)), start(sloc), pv)
+     invisible(list(sloc = sloc, pv = pv))
+ }
> geneTick = function(css, useSym=TRUE) {
+     annstr = css@annotation
+     require(annstr, character.only=TRUE)
+     anne = get(paste(gsub(".db", "", annstr), "CHRLOC", sep=""))
+     anneend = get(paste(gsub(".db", "", annstr), "CHRLOCEND", sep=""))
+     gsanne = get(paste(gsub(".db", "", annstr), "SYMBOL", sep=""))
+     ann = get
+     axis(3, at=abs(get(css@psid, anne)[1]),
+              labels=get(css@psid, gsanne)[1], col="green")
+     axis(3, at=abs(get(css@psid, anneend)[1]), col="red", labels="")
+ }
```

Here's an application.

```
> data(snpgr17)
> mplot(t1, snpgr17)
> geneTick(t1)
```



2 FDR and MAF

```
> library(ggtut)
> if (!exists("perm17")) perm17 = onePerm17ceu()
> perm17
```

```
eqtlTools results manager, computed Fri May  6 16:06:04 2011
gene annotation: illuminaHumanv1.db
There are 1 chromosomes analyzed.
some genes (out of 498): GI_10190685-S GI_10835020-S ... hmm23927-S hmm5188-S
some snps (out of 60967): rs6565733 rs1106175 ... rs7502145 rs4986109
```

We will obtain a realization of best per-gene eQTL scores under permutation of expression against genotype.

```
> probes = probesManaged(perm17,1)
> if (!exists("topperm"))
+ topperm = lapply(probes,
+    function(x)topFeats(probeId(x), mgr=perm17, ffind=1, n=1))
> if (!exists("toppermAt.1"))
+ toppermAt.1 = lapply(probes,
+    function(x)topFeats(probeId(x), mgr=perm17, ffind=1, n=150, minMAF=.1))
```

We need 150 candidates for the MAF-restricted run because these are sparse; topFeats will return an empty list if none of the top n scores meet the MAF threshold.

```
> if (!exists("obs17")) obs17 = observed17ceu()
> obs17

eqtlTools results manager, computed Fri May  6 16:05:50 2011
gene annotation: illuminaHumanv1.db
There are 1 chromosomes analyzed.
some genes (out of 498): GI_10190685-S GI_10835020-S ... hmm23927-S hmm5188-S
some snps (out of 60967): rs6565733 rs1106175 ... rs7502145 rs4986109

> if (!exists("topobs"))
+ topobs = lapply(probes,
+    function(x)topFeats(probeId(x), mgr=obs17, ffind=1, n=1))
> if (!exists("topobsAt.1"))
+ topobsAt.1 = lapply(probes,
+    function(x)topFeats(probeId(x), mgr=obs17, ffind=1, n=150, minMAF=.1))
```

Plug-in estimates of FDR corresponding to quantiles of the “permutation distribution” of the test statistic can be obtained as follows.

```
> topps = unlist(topperm)
> topos = unlist(topobs)
> permQ = quantile(topps, c(.95, .975, .99, .995))
> nfalse = sapply(permQ, function(x)sum(topps>x))
> nsig = sapply(permQ, function(x)sum(topos>x))
> options(digits=4)
> cbind(threshold=permQ,
+    permpctile=c(.95,.975,.99,.995)*100, nfalse, nsig, fdr=nfalse/nsig)

      threshold permpctile nfalse nsig    fdr
95%       23.53       95.0     25   45 0.5556
97.5%     26.25       97.5     13   30 0.4333
99%       30.75       99.0      5   22 0.2273
99.5%     32.24       99.5      3   20 0.1500
```

If we confine attention to SNP with MAFs exceeding some small quantity, we can reduce the FDR.

```
> topps = sapply(toppermAt.1, max)
> topos = sapply(topobsAt.1, max)
> permQ = quantile(topps, c(.95, .975, .99, .995))
> nfalse = sapply(permQ, function(x)sum(topps>x))
> nsig = sapply(permQ, function(x)sum(topos>x))
> cbind(threshold=permQ,
+    permpctile=c(.95,.975,.99,.995)*100, nfalse, nsig, fdr=nfalse/nsig)
```

```
      threshold permpctile nfalse nsig    fdr
95%       19.15       95.0     25   78 0.3205
97.5%     20.55       97.5     13   53 0.2453
99%       22.04       99.0      5   36 0.1389
99.5%     24.02       99.5      3   22 0.1364
```

3 Master regulators in cis?

Here we consider the prospect of identifying SNP that are associated with more than one gene on the same chromosome.

The following code runs on a single core laptop in about 20 minutes; the results are saved for you.

```
> sn = snpsManaged(obs17, 1)
```

```
> bestGenesPerSNP_obs <- lapply(sn,
+    function(x) topFeats(rsid(x), mgr=obs17, useSym=FALSE, ffind=1))
> bestGenesPerSNP_perm <- lapply(sn,
+    function(x) topFeats(rsid(x), mgr=perm17, useSym=FALSE, ffind=1))
```

We obtain the stored results via

```
> data(bestGenesPerSNP_perm)
> data(bestGenesPerSNP_obs)
```

Each of these objects is a list with 60967 elements; each element is the SNP-specific 10 best association scores over all 498 genes on chr17 filtered for use in the tutorial.

```
> bestGenesPerSNP_obs[1:3]
```

```
$rs6565733
 GI_4506130-S GI_42476207-S GI_44889413-S GI_37544934-S GI_33636720-S
        11.54         10.40          9.59          9.45          8.70
GI_38142463-S GI_21389406-S GI_20149678-S  GI_4503894-S GI_22094078-S
         8.01          7.97          7.91          7.84          7.78
```

```
$rs1106175
GI_21389406-S GI_22202610-S GI_13375890-A  GI_4503894-S GI_31377553-S
         9.13          9.08          8.62          8.45          8.32
 GI_4506130-S  GI_5453631-S GI_12545398-S GI_21237796-A GI_37544934-S
         7.82          7.67          7.55          7.55          7.52

$rs17054921
GI_30795213-S GI_20070234-S  GI_5902001-S GI_39780587-S GI_37544011-S
        11.21          5.77          5.70          5.37          5.34
GI_17939343-S GI_24415399-S GI_10835020-S GI_41281472-S GI_21361528-S
         4.41          4.10          4.03          3.50          3.47
```

To proceed with a simple assessment, we examine our permutation-based realization from the null distribution of scores for the second-highest scoring gene per SNP.

```
> p2 = sapply(bestGenesPerSNP_perm, "[", 2)
> quantile(p2, c(0.99, 0.995))
```

```
  99% 99.5%
14.50 15.71
```

```
> o2 = sapply(bestGenesPerSNP_obs, "[", 2)
> quantile(p2, c(0.999, 0.9999))
```

```
 99.9% 99.99%
 22.09  26.64
```

```
> sum(o2 > 26.6)
```

```
[1] 10
```

```
> sum(p2 > 26.6)
```

```
[1] 7
```

This is not a good situation. Again, inclusion of SNP with very low MAF is problematic. To filter them away, in the absence of high-level functions, we make use of summaries that are computed with `eqtlTests`.

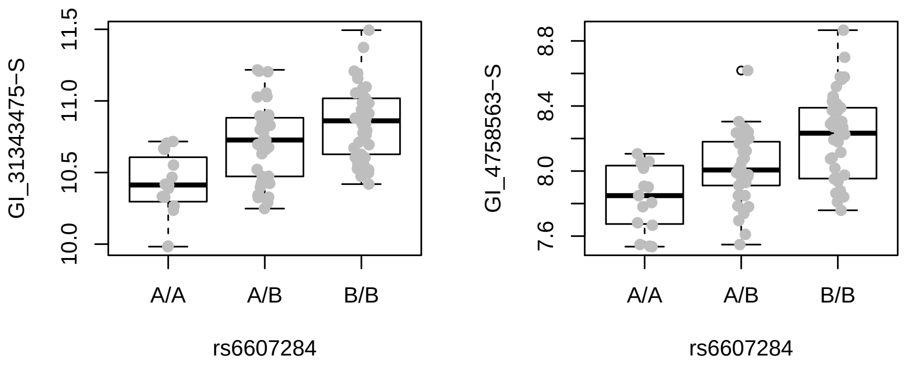
```
> MAF = function(mgr, ffind) mgr@summaryList[[ffind]][, "MAF"]/mgr@shortfac
> MAFS = MAF(perm17, 1)
> MAFS[1:5]
```

```
 rs6565733  rs1106175 rs17054921  rs8064924  rs8070440
      0.12       0.18       0.01       0.12       0.05
```

```
> lowmaf = names(MAFS)[MAFS < 0.1]
> p2rs = sapply(strsplit(names(p2), "\\."), "[[", 1)
> mm = match(lowmaf, p2rs)
> quantile(p2[-mm], c(0.999, 0.9999))

 99.9% 99.99%
 16.18  18.08

> quantile(o2[-mm], c(0.999, 0.9999))

 99.9% 99.99%
 18.72  20.23

> sum(p2[-mm] > 18.08)

[1] 5

> sum(o2[-mm] > 18.08)

[1] 66
```

So we can assert that there are about 66 SNP loci that are associated with at least 2 different genes (probes) at an FDR of about 8%. Let's look at one:

```
> sort(o2[-mm][o2[-mm] > 18.08], decreasing = TRUE)[1:5]

 rs6607284.GI_4758563-S rs12602297.GI_6031190-S rs4790175.GI_40255061-S
                  21.39                   20.90                   20.64
 rs781839.GI_40255061-S  rs781825.GI_40255061-S
                  20.23                   20.23

> 1 - pchisq(21, 1)

[1] 4.593e-06

> bestGenesPerSNP_obs[["rs6607284"]]

GI_31343475-S  GI_4758563-S GI_13376244-S GI_45505146-S GI_15147332-S
        22.41         21.39         19.40         18.99         18.37
GI_33519473-S GI_14249549-S GI_34850073-S GI_34147469-S  GI_8923770-S
        17.70         17.54         17.04         16.89         16.52

> c17 = getSS("GGdata", "17", renameChrs = "chr17")
> par(mfrow = c(2, 2))
> plot_EvG(probeId("GI_31343475-S"), rsid("rs6607284"), c17)
> plot_EvG(probeId("GI_4758563-S"), rsid("rs6607284"), c17)
```



Some annotation:

```
> library(illuminaHumanv1.db)
> get("GI_31343475-S", illuminaHumanv1GENENAME)

[1] "guanine nucleotide binding protein (G protein), alpha 13"

> get("GI_4758563-S", illuminaHumanv1GENENAME)

[1] "ubiquitin specific peptidase 6 (Tre-2 oncogene)"

> get("GI_31343475-S", illuminaHumanv1CHRLOC)

      17
-63005408

> get("GI_4758563-S", illuminaHumanv1CHRLOC)

     17
5031686
```

```
> get("GI_31343475-S", illuminaHumanv1PATH)

[1] "04270" "04730" "04810"

> get("GI_4758563-S", illuminaHumanv1PATH)

[1] NA
```

4 Session information

```
> sessionInfo()

R version 2.14.0 Under development (unstable) (2011-06-06 r56067)
Platform: x86_64-apple-darwin10.7.0/x86_64 (64-bit)

locale:
[1] C

attached base packages:
 [1] grid      splines   stats     graphics  grDevices datasets  utils
 [8] tools     methods   base

other attached packages:
 [1] multicore_0.1-5
 [2] VariantAnnotation_0.1.2
 [3] BSgenome.Hsapiens.UCSC.hg18_1.3.17
 [4] SNPlocs.Hsapiens.dbSNP.20090506_0.99.1
 [5] parody_1.11.0
 [6] hmyriB36_0.99.8
 [7] nlme_3.1-101
 [8] ggtut_0.0.25
 [9] Rsamtools_1.5.38
[10] ChIPpeakAnno_1.9.5
[11] gplots_2.8.0
[12] caTools_1.12
[13] gdata_2.8.2
[14] gtools_2.6.2
[15] limma_3.9.11
[16] GO.db_2.5.0
[17] BSgenome.Ecoli.NCBI.20080805_1.3.17
[18] BSgenome_1.21.3
[19] Biostrings_2.21.6
```

```
[20] multtest_2.9.0
[21] biomaRt_2.9.2
[22] GenomicFeatures_1.5.14
[23] GGdata_1.0.13
[24] illuminaHumanv1.db_1.10.0
[25] ff_2.2-2
[26] bit_1.1-7
[27] GGtools_3.11.32
[28] GenomicRanges_1.5.15
[29] org.Hs.eg.db_2.5.0
[30] rtracklayer_1.13.7
[31] RCurl_1.6-6
[32] bitops_1.0-4.1
[33] IRanges_1.11.11
[34] annotate_1.31.0
[35] AnnotationDbi_1.15.9
[36] GGBase_3.13.3
[37] RSQLite_0.9-4
[38] DBI_0.2-5
[39] snpStats_1.3.1
[40] Matrix_0.999375-50
[41] lattice_0.19-26
[42] survival_2.36-9
[43] Biobase_2.13.7
[44] BiocInstaller_1.1.12
[45] weaver_1.19.0
[46] codetools_0.2-8
[47] digest_0.5.0

loaded via a namespace (and not attached):
[1] MASS_7.3-13    XML_3.4-0      xtable_1.5-6   zlibbioc_0.1.6
```