Overview of *GGtools* for investigating genetics of gene expression

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1 Introduction

The *GGtools* package supports data analysis activities that link genotypic information such as SNP configurations to gene expression phenotype. We will attach the library and have a look at a basic demonstration resource.

```
> library(GGtools)
> data(chr20GGdem)
> class(chr20GGdem)

[1] "racExSet"
attr(,"package")
[1] "GGtools"

> chr20GGdem

racExSet instance (SNP rare allele count + expression)
rare allele count assayData:
  Storage mode: lockedEnvironment
  featureNames: rs4814683, rs6076506, ..., rs6062370, rs6090120 (117417 total)
  Dimensions:
           racs
Features 117417
Samples      58

expression assayData
  Storage mode: lockedEnvironment
  featureNames: 1007_s_at, 1053_at, ..., AFFX-r2-P1-cre-3_at, AFFX-r2-P1-cre-5_at (8793
  Dimensions:
        exprs
```

```
Features  8793
Samples     58

phenoData
An object of class "AnnotatedDataFrame"
  rowNames: NA06985, NA06993, ..., NA12892  (58 total)
  varLabels and varMetadata description:
    sample: hapmap id

Experiment data
  Experimenter name: Cheung VG
  Laboratory: Department of Pediatrics, University of Pennsylvania, Philadelphia, Penns
  Contact information:
  Title: Mapping determinants of human gene expression by regional and genome-wide asso
  URL:
  PMIDs: 16251966

  Abstract: A 180 word abstract is available. Use 'abstract' method.

Annotation [1] "hgfocus"
```

The racExSet class is an extension of the *eSet* class. It represents expression data from the hgfocus chip on 48 individuals in the CEU CEPH group, and SNP data obtained from their HapMap genotyping results.

The data are organized into an 8793 by 58 matrix of expression values accessible with the `exprs` method, and an 117417 by 58 of rare allele counts:

```
> dim(exprs(chr20GGdem))
```

```
[1] 8793   58
```

```
> dim(snps(chr20GGdem))
```

```
[1] 117417     58
```

```
> snps(chr20GGdem)[1:5, 1:5]
```

```
          NA06985 NA06993 NA06994 NA07000 NA07022
rs4814683       2       0       0       2       1
rs6076506       0       0       0       0      NA
rs6139074       2       0       0       2       1
rs1418258       2       0       0       2       1
rs7274499       0       0       0       0      NA
```

We need some genetic metadata about SNPs; these are derived from from SNP genotyping panels released on a chromosome-by-chromosome basis for CEPH participants by HapMap project:

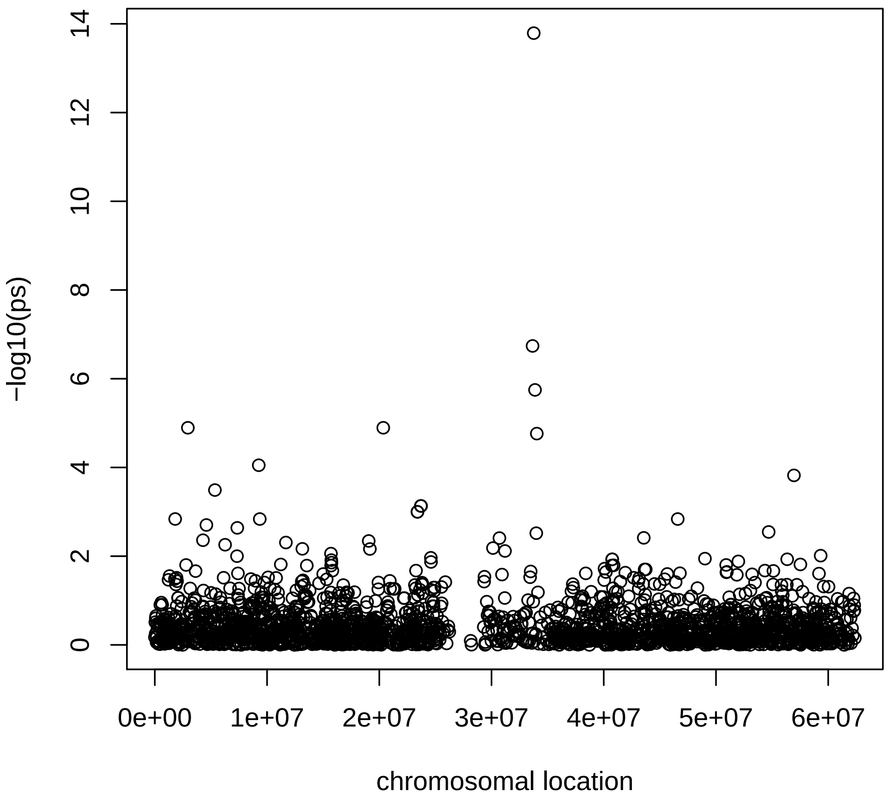
```
> data(chr20meta)
> chr20meta[1:4, ]

            pos strand
rs4814683  9795      +
rs6076506 11231      +
rs6139074 11244      +
rs1418258 11799      +
```

A basic task is to compute a screen (over the genome, or, more practically, if seeking interactive performance with commodity hardware, over a chromosome) of genotypic determination of expression. The `snpScreen` method helps with this; we illustrate an example related to results in Cheung and Spielman 2005:

```
> chr20GGdem = exclMono(chr20GGdem)
> S100 = snpScreen(chr20GGdem, chr20meta, genesym("CPNE1"), ~.,
+     lm, gran = 30)
> S100

GGtools snpScreenResult for call:
.local(racExSet = racExSet, snpMeta = snpMeta, gene = gene, formTemplate = formTemplate
    fitter = fitter, gran = gran)
There were 13 attempted fits,
and 13 were successful.
```

A primitive display is obtained as follows. We know that `lm` was used, so the relevant p-values are in the coefficient component of the summarized fit objects.

```
> ps = as.numeric(sapply(S100, function(x) try(summary(x)$coef[2,
+     4])))
> plot(S100@locs, -log10(ps), xlab = "chromosomal location")
```



2 Performance-oriented specialization

The snpScreen method illustrated above is very general (can accommodate and retain results of any R modeling function) but is fairly slow. We have added an R function fastAGM for fast fitting of an additive genetic model (equivalent to but much faster than using lm). This function is wrapped in an object, fastAGMfitter, to provide reflectance.

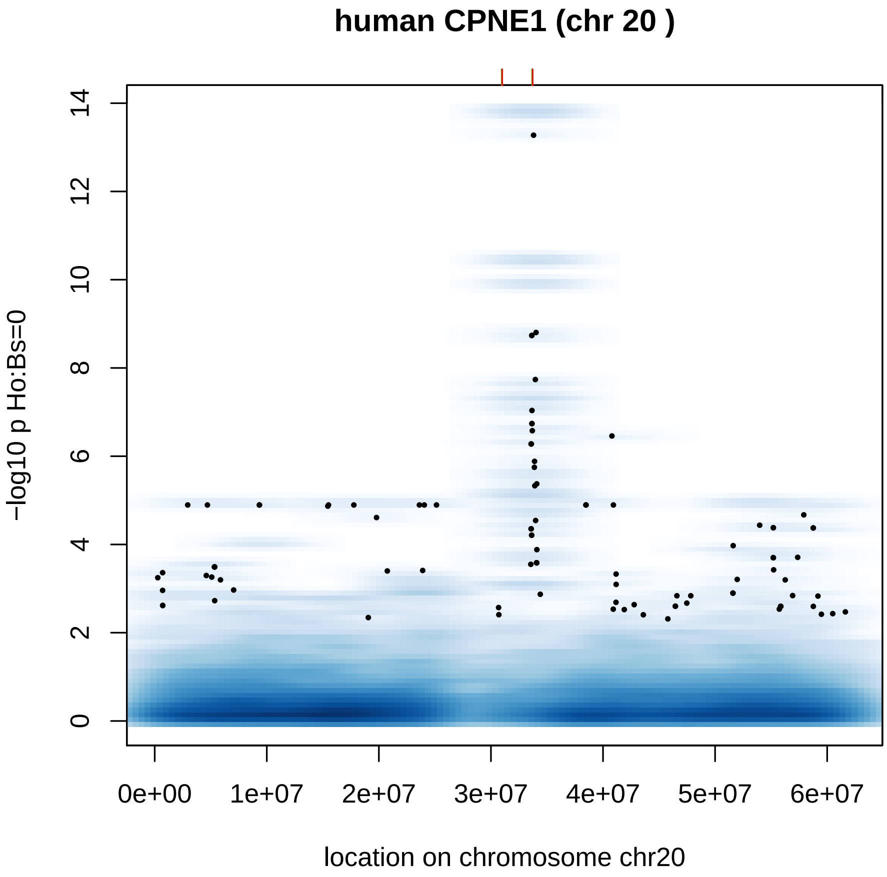
```
> ut = unix.time(sCPNE1 <- snpScreen(chr20GGdem, chr20meta, genesym("CPNE1"),
+     ~., fastAGMfitter))
> ut

   user  system elapsed 
  3.256   0.184   3.439 

> sCPNE1
```

```
GGtools snpScreenResult for call:
.local(racExSet = racExSet, snpMeta = snpMeta, gene = gene, formTemplate = formTemplate
    fitter = fitter, gran = gran)
There were 42442 attempted fits,
and 42442 were successful.
```

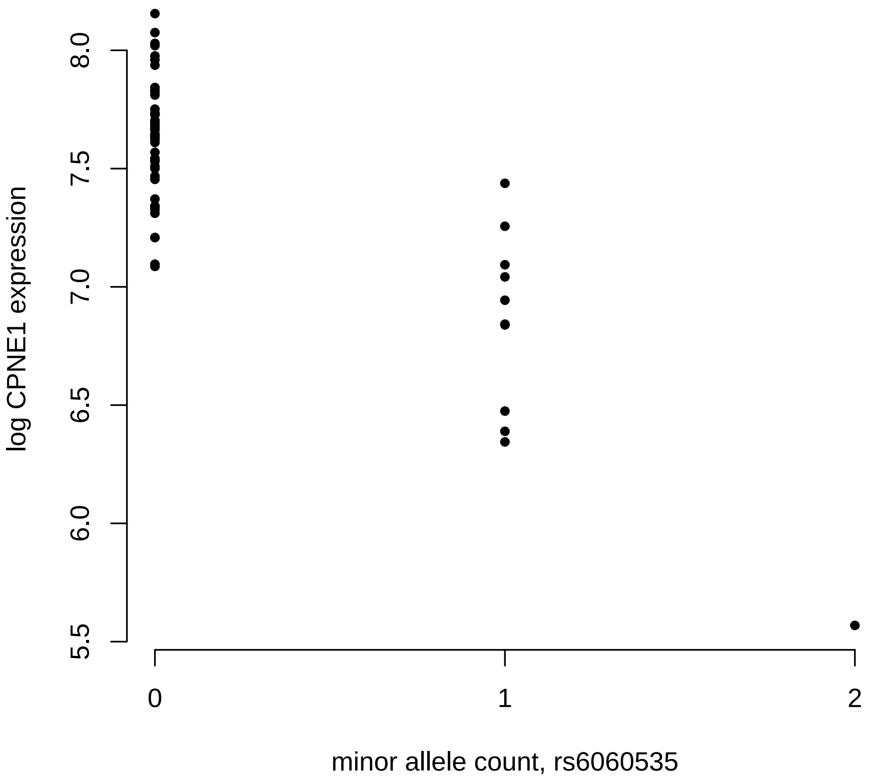
```
> wm = which.min(pp <- extract_p(sCPNE1))
> pp[wm]
```

```
   rs6058296
1.395072e-14
```

```
> data(geneLocs_hsa)
> plot_mlp(sCPNE1, chr20meta, geneLocDF = geneLocs_hsa)
```



```
> plot_EvG(chr20GGdem, genesym("CPNE1"), "rs6060535")
```



3 Creating new racExSet instances and supporting objects

Genotypes are available for a wide number of reference strains of mice through the Wellcome Trust; expression studies of such mice can be obtained using GEO. The data structure gse2031GG is an example:

```
> data(gse2031GG)
> gse2031GG

racExSet instance (SNP rare allele count + expression)
rare allele count assayData:
  Storage mode: lockedEnvironment
  featureNames: rs3683945, rs3707673, ..., rs4232414, mCV23022620 (13368 total)
  Dimensions:
          racs
```

```
Features 13368
Samples     44

expression assayData
  Storage mode: lockedEnvironment
  featureNames: 100001_at, 100002_at, ..., AFFX-b-ActinMur/M12481_M_at, AFFX-b-ActinMur
  Dimensions:
         exprs
Features 12488
Samples     44

phenoData
An object of class "AnnotatedDataFrame"
  rowNames: GSM36673, GSM36674, ..., GSM36716  (44 total)
  varLabels and varMetadata description:
    strain: strain from GEO soft file for series
    gsm: GEO id

Experiment data
  Experimenter name:
  Laboratory:
  Contact information:
  Title:
  URL:
  PMIDs:
  No abstract available.

Annotation [1] "mgu74av2"
```

The expression data are obtained by applying RMA to a collection of CEL files readily obtainable from GEO. The strains of the mice giving rise to the samples are obtained from the WebQTL ”contacts” link.

The genotype data for a large number of strains can be obtained from the INBREDS distribution at Wellcome Trust. The URL is `www.well.ox.ac.uk/mouse/INBREDS`. An example of the genotype data can be seen using the following code:

```
> ff = readLines(system.file("fileDemos/StrainInit.txt", package = "GGtools"))
> t(sapply(strsplit(ff[1:5], " "), function(x) x[c(1, 2, 3, 55,
+     56, 57, 101)]))
```

```
     [,1]        [,2]  [,3]       [,4]          [,5]          [,6]
[1,] "SNP"       "CHR" "POSITION" "AKXD-6/TyJ" "AKXD-7/TyJ" "AKXD-8/TyJ"
[2,] "rs3683945" "1"   "3157748"  "A"          "A"          "A"
```

```
[3,] "rs3707673" "1"   "3366533"  "G"          "G"          "G"
[4,] "rs6269442" "1"   "3451386"  "G"          "G"          "G"
[5,] "rs6336442" "1"   "3539826"  "G"          "G"          "G"
     [,7]
[1,] "BXA-12/PgnJ"
[2,] "A"
[3,] "G"
[4,] "A"
[5,] "A"
```

There are various representations provided in various places. Tightening the path to accurate genotyping data for reference strains would be useful.

Given an expression matrix and a genotyping file as provided at the INBREDS site, the INBREDSworkflow function will help generate a `racExSet` instance:

```
> args(INBREDSworkflow)

function (inbfile, emat, estrains, pd, mi, anno, fixup = NULL,
    fixchr = function(x) gsub("_random", "", x))
NULL
```

The `estrains` argument is a vector of strings defining the reference strains from which columns of `emat` were obtained through microarray hybridization. This vector will typically be available from a `phenoData` variable on the `ExpressionSet` from which the expression matrix was obtained. For the `gse2031GG`, this is the variable `strain`:

```
> as.character(gse2031GG$strain[1:5])

[1] "BXD6"  "BXD6"  "BXD8"  "BXD8"  "BXD11"
```

Now it appears that this nomenclature for strains is not always adopted directly. If we grep for a variation of BXD[nn] in the INBREDS strains file, we find

```
> grep("BXD-", strsplit(ff[1], " ")[[1]], value = TRUE)[1:5]

[1] "BXD-1/TyJ"  "BXD-11/TyJ" "BXD-12/TyJ" "BXD-13/TyJ" "BXD-14/TyJ"
```

and we see there are lexical variations introduced – we want BXD6, but the labels in the INBREDS file have hyphens. A `fixup` parameter allows inline reformatting of deviant strain identifiers as column names of the INBREDS file. Additionally, chromosome names are sometimes postpended with "-random", and some steps may be needed to simplify the chromosome nomenclature. The user must create/find the R code to obtain the desired results, or manually massage the source data files so that the desired regularities are present.

Once genotype codes are available with a strain naming convention that matches that of the expression samples, INBREDSworkflow will compute rare allele counts, bind the genotyping, expression, and phenotype data together, and generate a `racExSet` instance.

In summary, `make_racExSet` is a specification of materials that must be made mutually compatible for creation of a `GGtools` resource with which investigations of genetics of gene expression can be conveniently made. `HMworkflow` and `INBREDSworkflow` are utility functions that support this task for files coming from HapMap and Wellcome repositories.

4 Appendix: Package documentation for *GGtools*

```
                Information on package 'GGtools'

Description:



Package:       GGtools
Title:         software and data for genetical genomics (c) 2006 VJ
               Carey
Version:       1.6.1
Author:        Vince Carey <stvjc@channing.harvard.edu>
Description:   dealing with hapmap SNP reports, GWAS, etc.
Depends:       R (>= 2.5.0), methods, Biobase (>= 1.11.26), hgfocus,
               geneplotter(>= 1.11.8), mgu74av2
LazyData:      no
biocViews:     SNPsAndGeneticVariability, Genetics, Statistics
Maintainer:    Vince Carey <stvjc@channing.harvard.edu>
License:       Artistic (see COPYING)
Collate:       fastAGM.R snpMeta.R AllClasses.R AllGenerics.R
               HapMapUtils.R exclMono.R countRare.R genoString.R
               oneFit.R racExSet-methods.R snpScreenResult-methods.R
               snps3Pto.R updateObject.R Strains2rac.R wrapSNPmetaWh.R
               anno2chrbnd.R ogtes.R zzz.R
Built:         R 2.6.0; x86_64-unknown-linux-gnu; 2007-10-18 14:32:58;
               unix


Index:


GGfitter-class          Class "GGfitter" wrapper for special functions
                        to do whole genome screens
```

```
HM2rac                  compute rare allele count from a hapmap file
HMworkflow              function to bind together HapMap genotyping
                        results and expression data
Strains2rac             convert a Wellcome 'Strains' genotyping file to
                        rare allele count form
geneLocs                gene metadata from NCBI
genoStrings             create a character vector of genotype value
                        strings
make_racExSet           create a racExSet from simpler constituents
oGtypeExSet-class       Class "oGtypeExSet" ~~~
plot_EvG                plot expression vs genotype
racExSet-class          Class "racExSet" for combining RareAlleleCount
                        representations of SNPs, gene expression data,
                        and other phenotype data
snpMeta-class           Class "snpMeta" -- HapMap (or Wellcome INBREDS)
                        -based metadata structures for SNPs
snpScreen               compute model fits over a sequence of SNPs
snps                    accessor for genotype data in a ggExprSet


Further information is available in the following vignettes in
directory '/tmp/Rinst874332282/GGtools/doc':


GGoverview: GGtools overview (source)
```

Session information for this vignette build:

```
> sessionInfo()

R version 2.6.0 (2007-10-03)
x86_64-unknown-linux-gnu

locale:
LC_CTYPE=en_US;LC_NUMERIC=C;LC_TIME=en_US;LC_COLLATE=en_US;LC_MONETARY=en_US;LC_MESSAGE

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

other attached packages:
 [1] GGtools_1.6.1       mgu74av2_2.0.1      geneplotter_1.16.0
 [4] lattice_0.17-1      annotate_1.16.0     xtable_1.5-2
```

```
 [7] AnnotationDbi_1.0.6 RSQLite_0.6-3       DBI_0.2-4
[10] hgfocus_2.0.1       Biobase_1.16.1

loaded via a namespace (and not attached):
[1] grid_2.6.0         KernSmooth_2.22-21 RColorBrewer_1.0-1
```