

Causal Inference for QTL Networks with R/qtlnet Package

Elias Chaibub Neto and Brian S. Yandell

March 2, 2020

This vignette briefly describes the R/qtlnet package. This contains the legacy R/qdg package, and thus has code for Chaibub Neto et al. (2008) and Chaibub Neto et al. (2010) papers. Not all routines are described here. Further, the package has code for parallel processing using Condor that is not yet documented adequately.

R/qtlnet depends on R/pcalg, which in turn depends on RBGL from bioconductor. To ease your pain in installing, you can install as follows from within R:

```
> source("http://bioconductor.org/biocLite.R")
> biocLite("RBGL")
> install.packages("qtlnet")
```

This should work on any platform. It is possible to set up R so that it always checks Bioconductor or other repositories, using pull-down menu (Windows) or `.Rprofile` (see text below). See R package documentation for more information.

```
## .Rprofile using multiple repositories.
repos <- structure(c(CRAN="http://streaming.stat.iastate.edu/CRAN",
                     CRANextra="http://www.stats.ox.ac.uk/pub/RWin",
                     BioCsoft="http://www.bioconductor.org/packages/release/bioc",
                     Rforge="http://r-forge.r-project.org"))
options(repos=repos)
```

1 QTLNET routines

```
> library(qtlnet)
> options(stringsAsFactors = FALSE)
> default.stringsAsFactors()
```

```
[1] FALSE
```

Acyclic example:

```
> example(acyclic)

acyclc> ## Not run:
acyclc> ##D ## This reproduces Figure 1 exactly.
acyclc> ##D set.seed(3456789)
acyclc> ##D
acyclc> ##D tmp <- options(warn=-1)
acyclc> ##D acyclic.DG <- randomDAG(n = 100, prob = 2 / 99)
acyclc> ##D
acyclc> ##D options(tmp)
acyclc> ##D
acyclc> ##D ## Simulate cross object using R/qtl routines.
```

```

acyclc> ##D n.ind <- 300
acyclc> ##D mymap <- sim.map(len=rep(100,20), n.mar=10, eq.spacing=FALSE, include.x=FALSE)
acyclc> ##D mycross <- sim.cross(map=mymap, n.ind=n.ind, type="f2")
acyclc> ##D summary(mycross)
acyclc> ##D mycross <- sim.geno(mycross,n.draws=1)
acyclc> ##D
acyclc> ##D
acyclc> ##D ## Produce 100 QTL at three markers apiece.
acyclc> ##D acyclic.qtl <- generate.qtl.markers(cross=mycross,n.phe=100)
acyclc> ##D
acyclc> ##D ## Generate data from directed graph.
acyclc> ##D bp <- runif(100,0.5,1)
acyclc> ##D stdev <- runif(100,0.1,0.5)
acyclc> ##D bq <- matrix(0,100,3)
acyclc> ##D bq[,1] <- runif(100,0.2,0.4)
acyclc> ##D bq[,2] <- bq[,1]+0.1
acyclc> ##D bq[,3] <- bq[,2]+0.1
acyclc> ##D ## Generate phenotypes.
acyclc> ##D acyclic.data <- generate.qtl.pheno("acyclic", cross = mycross,
acyclc> ##D   bp = bp, bq = bq, stdev = stdev, allqtl = acyclic.qtl$allqtl)
acyclc> ##D
acyclc> ##D acyclic.qdg <- qdg(cross=acyclic.data,
acyclc> ##D   phenotype.names=paste("y",1:100,sep=""),
acyclc> ##D   marker.names=acyclic.qtl$markers,
acyclc> ##D   QTL=acyclic.qtl$allqtl,
acyclc> ##D   alpha=0.005,
acyclc> ##D   n.qdg.random.starts=1,
acyclc> ##D   skel.method="pcskel")
acyclc> ##D save(acyclic.DG, acyclic.qtl, acyclic.data, acyclic.qdg,
acyclc> ##D   file = "acyclic.RData", compress = TRUE)
acyclc> ## End(Not run)
acyclc>
acyclc> data(acyclic)

acyclc> dims <- dim(acyclic.data$pheno)

acyclc> SuffStat <- list(C = cor(acyclic.data$pheno), n = dims[1])

acyclc> pc <- skeleton(SuffStat, gaussCIttest, p = dims[2], alpha = 0.005)

acyclc> summary(pc)
Object of class 'pcAlgo', from Call:
skeleton(suffStat = SuffStat, indepTest = gaussCIttest, alpha = 0.005,
  p = dims[2])

Nmb. edgetests during skeleton estimation:
=====
Max. order of algorithm: 3
Number of edgetests from m = 0 up to m = 3 : 5426 3690 384 36

Graphical properties of skeleton:
=====
Max. number of neighbours: 4 at node(s) 1 4 19 50 63 65 69 70 78
Avg. number of neighbours: 1.86

```

Adjacency Matrix G:

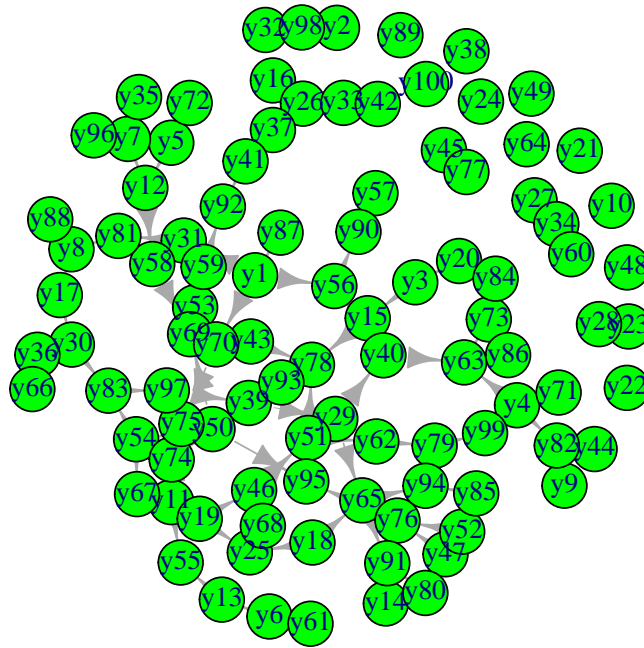
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1
2
3	1	1
4
5	1
6	1
7	1
8	1
9
10
11	1
12	1	.	1
13	1
14
15	.	.	1
16	1	.	.
17	1
18	1	.	.
19	1	1	.	.
20	.	.	1
21
22
23	1
24
25	1	1
26	1
27
28	1
29
30	1
31	1
32
33	1	.	.
34	1	.
35	1
36
37	1	.	.
38
39
40
41
42
43
44
45
46	1
47
48
49
50
51

[illegible]


```
acyclc> summary(graph.qdg(acyclc.qdg))
IGRAPH 8fa330b DN-- 259 394 --
+ attr: name (v/c), label (v/c), color (v/c), fill (v/c), width (e/n)

acyclc> gr <- graph.qdg(acyclc.qdg, include.qtl = FALSE)

acyclc> plot(gr)
```



Cyclic A example:

```
> example(cyclica)

cyclic> ## Not run:
cyclic> ##D bp <- matrix(0, 6, 6)
cyclic> ##D bp[2,1] <- bp[4,2] <- bp[4,3] <- bp[5,4] <- bp[2,5] <- bp[6,5] <- 0.5
cyclic> ##D stdev <- rep(0.025, 6)
cyclic> ##D
cyclic> ##D ## Use R/qlt routines to simulate.
cyclic> ##D set.seed(3456789)
cyclic> ##D mymap <- sim.map(len = rep(100,20), n.mar = 10, eq.spacing = FALSE,
cyclic> ##D   include.x = FALSE)
cyclic> ##D mycross <- sim.cross(map = mymap, n.ind = 200, type = "f2")
cyclic> ##D mycross <- sim.geno(mycross, n.draws = 1)
cyclic> ##D
cyclic> ##D cyclica.qlt <- generate.qlt.markers(cross = mycross, n.phe = 6)
cyclic> ##D mygeno <- pull.geno(mycross)[, unlist(cyclica.qlt$markers)]
cyclic> ##D
cyclic> ##D cyclica.data <- generate.qlt.pheno("cyclica", cross = mycross, burnin = 2000,
cyclic> ##D   bq = c(0.2,0.3,0.4), bp = bp, stdev = stdev, geno = mygeno)
cyclic> ##D save(cyclica.qlt, cyclica.data, file = "cyclica.RData", compress = TRUE)
cyclic> ## End(Not run)
```

```

cyclic>
cyclic> data(cyclica)

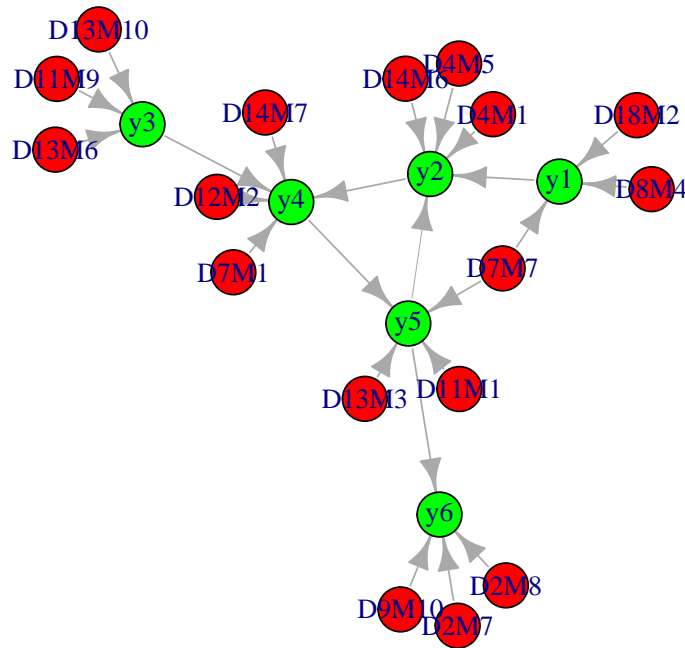
cyclic> out <- qdg(cross=cyclica.data,
cyclic+           phenotype.names=paste("y",1:6,sep=""),
cyclic+           marker.names=cyclica.qtl$markers,
cyclic+           QTL=cyclica.qtl$allqtl,
cyclic+           alpha=0.005,
cyclic+           n.qdg.random.starts=10,
cyclic+           skel.method="pcskel")

cyclic> gr <- graph.qdg(out)

cyclic> gr
IGRAPH d606b74 DN-- 23 24 --
+ attr: name (v/c), label (v/c), color (v/c), fill (v/c), width (e/n)
+ edges from d606b74 (vertex names):
  [1] y1    ->y2 y2    ->y4 y5    ->y2 y3    ->y4 y4    ->y5 y5    ->y6
  [7] D18M2 ->y1 D8M4   ->y1 D7M7   ->y1 D4M5   ->y2 D4M1   ->y2 D14M6 ->y2
 [13] D13M6 ->y3 D11M9 ->y3 D13M10->y3 D12M2 ->y4 D7M1   ->y4 D14M7 ->y4
 [19] D7M7   ->y5 D13M3 ->y5 D11M1 ->y5 D2M8   ->y6 D2M7   ->y6 D9M10 ->y6

cyclic> plot(gr)

```



Cyclic B example:

```
> example(cyclcb)
```

```
cyclcb> ## Not run:
cyclcb> ##D bp <- matrix(0, 6, 6)
cyclcb> ##D bp[2,1] <- bp[1,5] <- bp[3,1] <- bp[4,2] <- bp[5,4] <- bp[5,6] <- bp[6,3] <- 0.5
cyclcb> ##D stdev <- rep(0.025, 6)
cyclcb> ##D
cyclcb> ##D ## Use R/qlt routines to simulate.
cyclcb> ##D set.seed(3456789)
cyclcb> ##D mymap <- sim.map(len = rep(100,20), n.mar = 10, eq.spacing = FALSE,
cyclcb> ##D   include.x = FALSE)
cyclcb> ##D mycross <- sim.cross(map = mymap, n.ind = 200, type = "f2")
cyclcb> ##D mycross <- sim.geno(mycross, n.draws = 1)
cyclcb> ##D
cyclcb> ##D cyclcb.qlt <- generate.qlt.markers(cross = mycross, n.phe = 6)
cyclcb> ##D mygeno <- pull.geno(mycross)[, unlist(cyclcb.qlt$markers)]
cyclcb> ##D
cyclcb> ##D cyclcb.data <- generate.qlt.pheno("cyclcb", cross = mycross, burnin = 2000,
cyclcb> ##D   bq = c(0.2,0.3,0.4), bp = bp, stdev = stdev, geno = mygeno)
cyclcb> ##D save(cyclcb.qlt, cyclcb.data, file = "cyclcb.RData", compress = TRUE)
cyclcb> ## End(Not run)
```

```

cyclcb>
cyclcb> data(cyclcb)

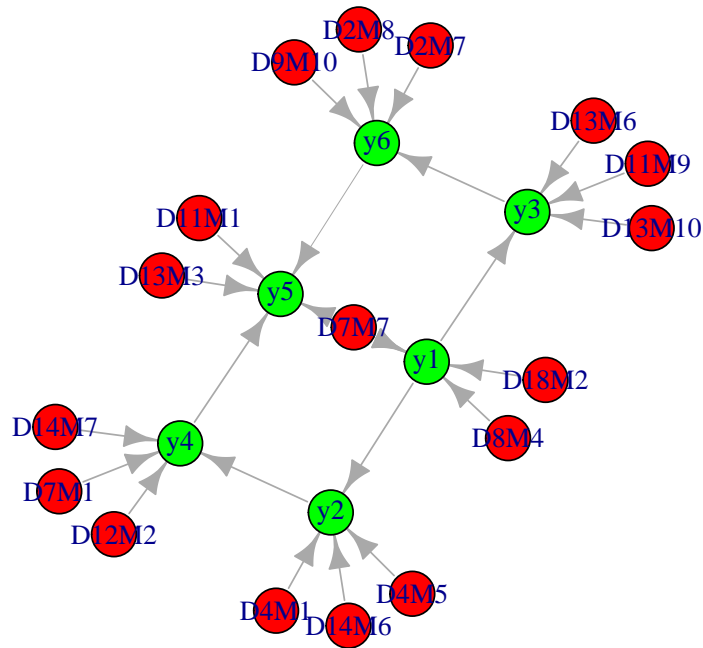
cyclcb> out <- qdg(cross=cyclcb.data,
cyclcb+           phenotype.names=paste("y",1:6,sep=""),
cyclcb+           marker.names=cyclcb.qtl$markers,
cyclcb+           QTL=cyclcb.qtl$allqtl,
cyclcb+           alpha=0.005,
cyclcb+           n.qdg.random.starts=10,
cyclcb+           skel.method="pcskel")

cyclcb> gr <- graph.qdg(out)

cyclcb> gr
IGRAPH 9db784e DN-- 23 25 --
+ attr: name (v/c), label (v/c), color (v/c), fill (v/c), width (e/n)
+ edges from 9db784e (vertex names):
  [1] y1    ->y2 y1    ->y3 y5    ->y1 y2    ->y4 y3    ->y6 y4    ->y5
  [7] y6    ->y5 D18M2 ->y1 D8M4  ->y1 D7M7  ->y1 D4M5  ->y2 D4M1  ->y2
 [13] D14M6 ->y2 D13M6 ->y3 D11M9 ->y3 D13M10->y3 D12M2 ->y4 D7M1  ->y4
 [19] D14M7 ->y4 D7M7  ->y5 D13M3 ->y5 D11M1 ->y5 D2M8  ->y6 D2M7  ->y6
 [25] D9M10 ->y6

cyclcb> plot(gr)

```



Cyclic C example:

```
> example(cyclicc)

cyclcc> ## Not run:
cyclcc> ##D bp <- matrix(0, 6, 6)
cyclcc> ##D bp[2,5] <- 0.5
cyclcc> ##D bp[5,2] <- 0.8
cyclcc> ##D bp[2,1] <- bp[3,2] <- bp[5,4] <- bp[6,5] <- 0.5
cyclcc> ##D stdev <- rep(0.025, 6)
cyclcc> ##D
cyclcc> ##D ## Use R/qlt routines to simulate map and genotypes.
cyclcc> ##D set.seed(34567899)
cyclcc> ##D mymap <- sim.map(len = rep(100,20), n.mar = 10, eq.spacing = FALSE,
cyclcc> ##D   include.x = FALSE)
cyclcc> ##D mycross <- sim.cross(map = mymap, n.ind = 200, type = "f2")
cyclcc> ##D mycross <- sim.geno(mycross, n.draws = 1)
cyclcc> ##D
cyclcc> ##D ## Use R/qdg routines to produce QTL sample and generate phenotypes.
cyclcc> ##D cyclicc.qlt <- generate.qlt.markers(cross = mycross, n.phe = 6)
cyclcc> ##D mygeno <- pull.geno(mycross)[, unlist(cyclicc.qlt$markers)]
cyclcc> ##D
cyclcc> ##D cyclicc.data <- generate.qlt.pheno("cyclicc", cross = mycross, burnin = 2000,
```

```

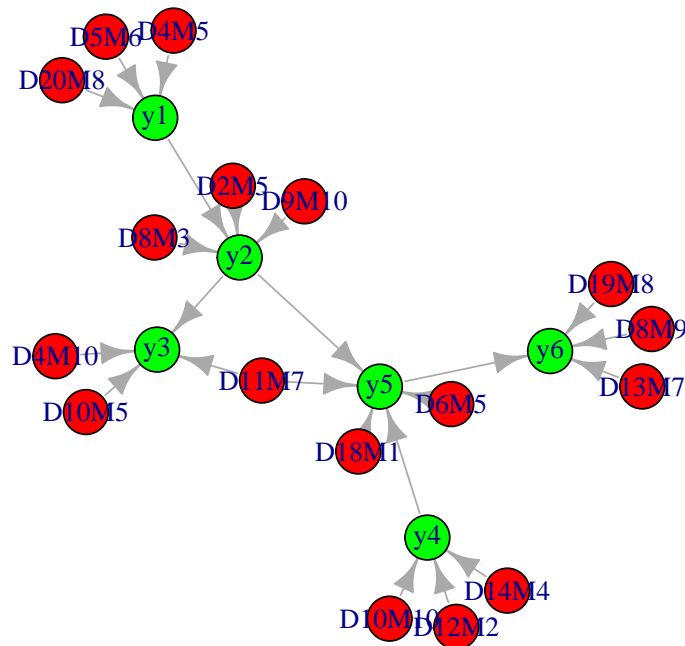
cyclcc> ##D   bq = c(0.2,0.3,0.4), bp = bp, stdev = stdev, geno = mygeno)
cyclcc> ##D save(cyclicc.qtl, cyclicc.data, file = "cyclicc.RData", compress = TRUE)
cyclcc> ## End(Not run)
cyclcc>
cyclcc> data(cyclicc)

cyclcc> out <- qdg(cross=cyclicc.data,
cyclcc+           phenotype.names=paste("y",1:6,sep=""),
cyclcc+           marker.names=cyclicc.qtl$markers,
cyclcc+           QTL=cyclicc.qtl$allqtl,
cyclcc+           alpha=0.005,
cyclcc+           n.qdg.random.starts=1,
cyclcc+           skel.method="pcskel")

cyclcc> gr <- graph.qdg(out)

cyclcc> plot(gr)

```



GLX network example (from Chaibub Neto et al. (2008)):

```

> example(glxnet)
glxnet> data(glxnet)

```



```

glxnet> glxnet.cross <- calc.genoprob(glxnet.cross)

glxnet> set.seed(1234)

glxnet> glxnet.cross <- sim.geno(glxnet.cross)

glxnet> n.node <- nphe(glxnet.cross) - 2 ## Last two are age and sex.

glxnet> markers <- glxnet.qtl <- vector("list", n.node)

glxnet> for(i in 1:n.node) {
glxnet+   ac <- model.matrix(~ age + sex, glxnet.cross$pheno)[, -1]
glxnet+   ss <- summary(scanone(glxnet.cross, pheno.col = i,
glxnet+                         addcovar = ac, intcovar = ac[,2]),
glxnet+                         threshold = 2.999)
glxnet+   glxnet.qtl[[i]] <- makeqtl(glxnet.cross, chr = ss$chr, pos = ss$pos)
glxnet+   markers[[i]] <- find.marker(glxnet.cross, chr = ss$chr, pos = ss$pos)
glxnet+ }

glxnet> names(glxnet.qtl) <- names(markers) <- names(glxnet.cross$pheno)[seq(n.node)]

glxnet> glxnet.qdg <- qdg(cross=glxnet.cross,
glxnet+   phenotype.names = names(glxnet.cross$pheno[,seq(n.node)]),
glxnet+   marker.names = markers,
glxnet+   QTL = glxnet.qtl,
glxnet+   alpha = 0.05,
glxnet+   n.qdg.random.starts=10,
glxnet+   addcov="age",
glxnet+   intcov="sex",
glxnet+   skel.method="udgskel",
glxnet+   udg.order=6)

glxnet> glxnet.qdg
$UDG
      node1  node2 edge
1      Glx Slc38a3   0
2      Glx   Ivd   0
3      Glx Slc1a2   1
4      Glx   Ass1   0
5      Glx   Arg1   0
6      Glx   Pck1   0
7      Glx   Agxt   1
8 Slc38a3   Ivd   0
9 Slc38a3 Slc1a2   0
10 Slc38a3   Ass1   0
11 Slc38a3   Arg1   0
12 Slc38a3   Pck1   0
13 Slc38a3   Agxt   0
14   Ivd Slc1a2   1
15   Ivd   Ass1   0
16   Ivd   Arg1   0
17   Ivd   Pck1   0
18   Ivd   Agxt   1
19 Slc1a2   Ass1   0

```

20	Slc1a2	Arg1	0
21	Slc1a2	Pck1	0
22	Slc1a2	Agxt	0
23	Ass1	Arg1	0
24	Ass1	Pck1	0
25	Ass1	Agxt	0
26	Arg1	Pck1	1
27	Arg1	Agxt	1
28	Pck1	Agxt	0

\$DG

	node1	direction	node2	lod	score
1	Glx	---->	Slc1a2	0.3464680	
2	Glx	---->	Agxt	1.5834015	
3	Ivd	---->	Slc1a2	2.5655168	
4	Ivd	---->	Agxt	1.8999843	
5	Arg1	<----	Pck1	-0.3165180	
6	Arg1	<----	Agxt	-0.5102432	

\$best.lm

[1] 1

\$\$Solutions

\$\$Solutions\$solutions

\$\$Solutions\$solutions[[1]]

	node1	direction	node2	lod
1	Glx	---->	Slc1a2	0.08870972
2	Glx	---->	Agxt	1.20241212
3	Ivd	---->	Slc1a2	2.30775847
4	Ivd	---->	Agxt	1.51899498
5	Arg1	---->	Pck1	1.60774597
6	Arg1	<----	Agxt	-2.02572245

\$\$Solutions\$loglikelihood

[1] 280.6703

\$\$Solutions\$BIC

[1] 15.24228

\$marker.names

\$marker.names\$Glx

[1] "D2Mit51" "D4Mit190" "D5Mit183" "D7Mit117" "D9Mit182" "D13Mit76"

\$marker.names\$Slc38a3

[1] "D8Mit45"

\$marker.names\$Ivd

[1] "D2Mit106" "D8Mit45" "D13Mit91"

\$marker.names\$Slc1a2

[1] "D2Mit395" "D9Mit20" "D18Mit177"

```

$marker.names$Ass1
[1] "D2Mit263" "D4Mit190" "D5Mit240" "D8Mit249" "D15Mit252"

$marker.names$Arg1
[1] "D1Mit64" "D2Mit263" "D9Mit207"

$marker.names$Pck1
[1] "D4Mit37" "D10Mit233"

$marker.names$Agxt
[1] "D2Mit411" "D7Mit294" "D14Mit126"

$phenotype.names
[1] "Glx" "Slc38a3" "Ivd" "Slc1a2" "Ass1" "Arg1" "Pck1"
[8] "Agxt"

$addcov
[1] "age"

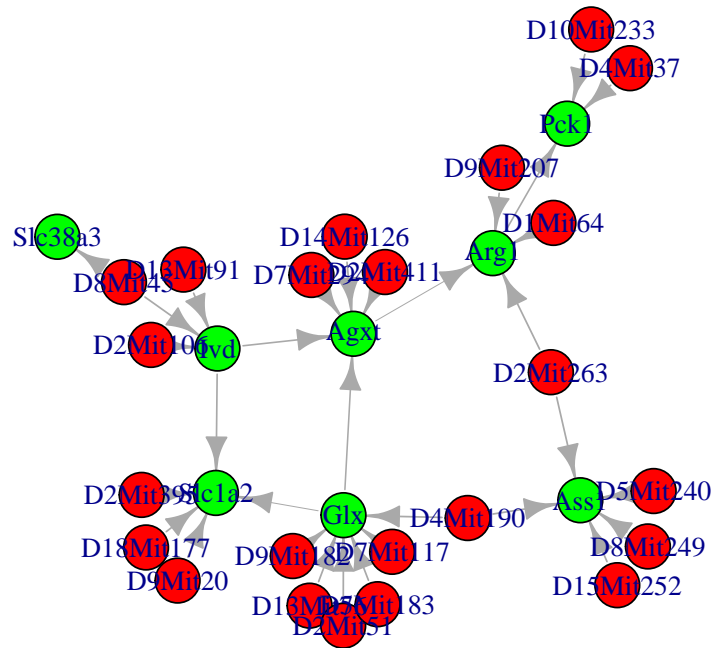
attr("class")
[1] "qdg" "list"

glxnet> gr <- graph.qdg(glxnet.qdg)

glxnet> plot(gr)

glxnet> ## Or use tkplot().
glxnet> ## Not run:
glxnet> ##D glxnet.cross <- clean(glxnet.cross)
glxnet> ##D save(glxnet.cross, glxnet.qdg, glxnet.qtl, file = "glxnet.RData", compress = TRUE)
glxnet> ## End(Not run)
glxnet>
glxnet>
glxnet>

```



2 QDG routines

The QDG routines are now incorporated into R/qtlnet. This document shows how to generate data, fit a QDG model and plot the inferred graph. We focus on a simple graph, $y_1 \rightarrow y_3$, $y_2 \rightarrow y_3$ and $y_3 \rightarrow y_4$, with QTLs that affect each of the three phenotypes.

```
> library(qtlnet)
```

Simulate a genetic map (20 autosomes, 10 not equally spaced markers per chromosome).

```
> mymap <- sim.map(len=rep(100,20), n.mar=10, eq.spacing=FALSE, include.x=FALSE)
```

Simulate an F2 cross object with n.ind (number of individuals).

```
> n.ind <- 200
```

```
> mycross <- sim.cross(map=mymap, n.ind=n.ind, type="f2")
```

Produce multiple imputations of genotypes using the sim.geno function. The makeqtl function requires it, even though we are doing only one imputation (since we don't have missing data and we are using the genotypes in the markers, one imputation is enough).

```
> mycross <- sim.geno(mycross, n.draws=1)
```

Use 2 markers per phenotype, samples from the cross.

```
> genotypes <- pull.geno(mycross)
> geno.names <- dimnames(genotypes)[[2]]
> m1 <- sample(geno.names,2,replace=FALSE)
> m2 <- sample(geno.names,2,replace=FALSE)
> m3 <- sample(geno.names,2,replace=FALSE)
> m4 <- sample(geno.names,2,replace=FALSE)
> ## get marker genotypes
> g11 <- genotypes[,m1[1]]; g12 <- genotypes[,m1[2]]
> g21 <- genotypes[,m2[1]]; g22 <- genotypes[,m2[2]]
> g31 <- genotypes[,m3[1]]; g32 <- genotypes[,m3[2]]
> g41 <- genotypes[,m4[1]]; g42 <- genotypes[,m4[2]]
> ## generate phenotypes
> y1 <- runif(3,0.5,1)[g11] + runif(3,0.5,1)[g12] + rnorm(n.ind)
> y2 <- runif(3,0.5,1)[g21] + runif(3,0.5,1)[g22] + rnorm(n.ind)
> y3 <- runif(1,0.5,1) * y1 + runif(1,0.5,1) * y2 + runif(3,0.5,1)[g31] + runif(3,0.5,1)[g32] + rnorm(1)
> y4 <- runif(1,0.5,1) * y3 + runif(3,0.5,1)[g41] + runif(3,0.5,1)[g42] + rnorm(n.ind)
```

Incorporate phenotypes into cross object.

```
> mycross$pheno <- data.frame(y1,y2,y3,y4)
```

Create markers list.

```
> markers <- list(m1,m2,m3,m4)
> names(markers) <- c("y1","y2","y3","y4")
```

Create qtl object.

```
> allqtls <- list()
> m1.pos <- find.markerpos(mycross, m1)
> allqtls[[1]] <- makeqtl(mycross, chr = m1.pos[, "chr"], pos = m1.pos[, "pos"])
> m2.pos <- find.markerpos(mycross, m2)
> allqtls[[2]] <- makeqtl(mycross, chr = m2.pos[, "chr"], pos = m2.pos[, "pos"])
> m3.pos <- find.markerpos(mycross, m3)
> allqtls[[3]] <- makeqtl(mycross, chr = m3.pos[, "chr"], pos = m3.pos[, "pos"])
> m4.pos <- find.markerpos(mycross, m4)
> allqtls[[4]] <- makeqtl(mycross, chr = m4.pos[, "chr"], pos = m4.pos[, "pos"])
> names(allqtls) <- c("y1","y2","y3","y4")
```

Infer QDG object.

```
> out <- qdg(cross=mycross,
+           phenotype.names = c("y1","y2","y3","y4"),
+           marker.names = markers,
+           QTL = allqtls,
+           alpha = 0.005,
+           n.qdg.random.starts=10,
+           skel.method="pcskel")
> out
```

\$UDG

	node1	node2	edge
1	y1	y3	1
2	y2	y3	1

```

5   y3   y4   1

$DG
  node1 direction node2   lod score
1   y1     <----   y3 -0.32735729
2   y2     ---->   y3  0.04608156
3   y3     ---->   y4  1.54554440

$best.lm
[1] 1

$Solutions
$Solutions$solutions
$Solutions$solutions[[1]]
  node1 direction node2      lod
1   y1     ---->   y3  2.251699
2   y2     ---->   y3  2.625138
3   y3     ---->   y4 15.839739

$Solutions$loglikelihood
[1] -1104.19

$Solutions$BIC
[1] 2351.435

$marker.names
$marker.names$y1
[1] "D14M5" "D3M10"

$marker.names$y2
[1] "D9M8" "D5M8"

$marker.names$y3
[1] "D12M1" "D18M2"

$marker.names$y4
[1] "D7M4" "D19M1"

$phenotype.names
[1] "y1" "y2" "y3" "y4"

attr(,"class")
[1] "qdg" "list"

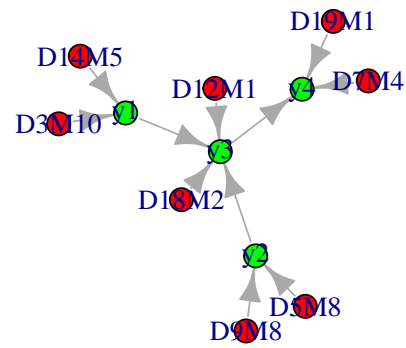
```

Plot object. The graph is an object of class `igraph`, which can be plotted using the `igraph` package.

```

> graph <- graph.qdg(out)
> plot(graph)

```



You can use `tkplot()` for an interactive plot.