R implementation

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↰ Project started Dec 10 2017

Loaded functions:

```
#source("/media/Data/Dropbox/humanR/01funcs.R")
rm(list=ls())
#setwd("/media/Data/Dropbox/humanR/PD/")
#setwd("~/Dropbox/humanR/PD/")
###load("PD.Rdata", .GlobalEnv)
#lsos(pat="")
```

Load packages.

```
pkgs <- c('gdata','caret','leaps','glmnet','lattice','latticeExtra',
          'ggplot2', 'dplyr', 'tidyr', 'RColorBrewer','igraph',
          'DescTools')
lapply(pkgs, require, character.only = TRUE)

Warning in library(package, lib.loc = lib.loc, character.only = TRUE, logical.return
= TRUE, :  there is no package called 'caret'
Warning in library(package, lib.loc = lib.loc, character.only = TRUE, logical.return
= TRUE, :  there is no package called 'glmnet'
```

1 Data structure

Data is from patients with Lymphoma tumors, either undergone or not a Rituximab CHOP treatment. Some patients show relapse after treatment. Tumors migrate though nodal (lymphnodes) or extranodal tissues. Tumors involve two different subtypes of cells of origin, ABC or GCB. **The first aim is to find correlation genes that respond differently to treatment, nodal transmission, and cell subtypes.**

```
metadata <- read.table("data/phenodata", sep = "\t", header = T)
```

```
head(metadata)
```

```
  SAMPLE_ID PATIENT_ID Timepoint OTHER_ID   res_id INCLUDE_MATCHING
1 CNR1001T1    CNR1001        T1          01-18186              YES
2 CNR1002T1    CNR1002        T1          01-26575              YES
3 CNR1002T2    CNR1002        T2          01-26575              YES
4 CNR1003T1    CNR1003        T1          02-10117              YES
5 CNR1006T1    CNR1006        T1 DLC_0304 03-11110              YES
6 CNR1007T1    CNR1007        T1 DLC_0193 03-26640              YES
  INCLUDED_SUBMISSION_TCAG             GROUP SITE Normalization Score
1                      YES CNS_RELAPSE_RCHOP   SO            37   789
2                      YES CNS_RELAPSE_RCHOP   GA            60  3548
3                      YES CNS_RELAPSE_RCHOP  CNS            62  3941
4                      YES CNS_RELAPSE_RCHOP   SO            79  -355
5                      YES CNS_RELAPSE_RCHOP   LN           843  -245
6                      YES CNS_RELAPSE_RCHOP   SO           143  3469
  ABClikelihood Prediction BCL2_BA BCL6_BA MYC_BA DH COMMENT CODE_OS
1             0        GCB       0       0      1  0               1
2             1        ABC       0       0      0  0               1
3             1        ABC       0       0      0  0               1
4             0        GCB       1       1      1  1               1
5             0        GCB       1       0      0  0               1
6             1        ABC       0       0      0  0               1
  CODE_DSS CODE_PFS CODE_TTP CODE_CNS Overall.survival..y.
1        1        1        1        1                 0.87
2        1        1        1        1                 2.98
3        1        1        1        1                 2.98
4        1        1        1        1                 0.60
5        1        1        1        1                 0.42
6        1        1        1        1                 4.64
  Disease.specific.survival..y. Progression.free.survival..y.
1                          0.87                          0.52
2                          2.98                          0.38
3                          2.98                          0.38
4                          0.60                          0.31
5                          0.42                          0.13
6                          4.64                          0.54
  Time.to.progression..y. Time.to.CNS.relapse..y. SEX AGE STAGE
1                    0.52                    0.52   F  82    4B
2                    0.38                    0.38   F  77    4A
3                    0.38                    0.38   F  77    4A
4                    0.31                    0.31   F  54    4A
5                    0.13                    0.15   M  59   2BE
6                    0.54                    0.45   M  62   1AE
  STAGEGRP E4SITE PS LDH LDHNORML LDHRATIO MASS IPI IPI_GROUP
1      ADV   BoSo  0 997      415     2.40   14   4         3
2      ADV   GaKi  1  -1      210    -1.00    1  -1         2
3      ADV   GaKi  1  -1      210    -1.00    1  -1         2
4      ADV SoOvUt  4 993      210     4.73   11   4         3
5      ADV     Gi  2 861      540     1.59    5   2         2
6      LIM   BoSo  1 424      210     2.02    7   3         2
  CNS.RiskScore CNS.RiskGrp Rehyb
1             4           3    NO
2            -1          -1   YES
3            -1          -1   YES
4             4           3    NO
5             2           2    NO
6             3           2    NO
```

In the first steps of the analysis, the samples will be classified (supervised) into the following categories.

```
metadata <- read.table("data/phenodata", sep = "\t", header = T) %>%
    dplyr::select(SAMPLE_ID, Timepoint, GROUP, SITE, Score, Prediction, ABClikelihood) %>%
    filter(Timepoint != "T2") %>%
    mutate(Groups = case_when(GROUP %in% c("CNS_RELAPSE_RCHOP",
                                            "CNS_RELAPSE_CHOPorEQUIVALENT",
                                            "CNS_DIAGNOSIS") ~ "CNS",
                               GROUP %in% c("TESTICULAR_NO_CNS_RELAPSE", "NO_RELAPSE") ~ "NOREL",
                               GROUP == "SYTEMIC_RELAPSE_NO_CNS" ~ "SYST",
                               TRUE ~ "CTRL")) %>%
    mutate(ABClassify = case_when(ABClikelihood >= .9 ~ "ABC",
                                  ABClikelihood <= .1 ~ "GCB",
                                  TRUE ~ "U")) %>%
    mutate(ABCScore = case_when(Score > 2412 ~ "ABC",
                                Score <= 1900 ~ "GCB",
#                                Score == NA ~ "NA",
                                TRUE ~ "U")) %>%
    mutate(Nodes = case_when(SITE == "LN" ~ "LN",
                             SITE == "TO" ~ "LN",
                             SITE == "SP" ~ "LN",
                             TRUE ~ "EN")) %>%
    mutate(Lymphnodes = case_when(Nodes == "LN" ~ 1, TRUE ~ 0))

# make sure all samples preserve their ID
metadata$Groups <- as.factor(metadata$Groups)
metadata$ABClassify <- as.factor(metadata$ABClassify)
metadata$ABCScore <- as.factor(metadata$ABCScore)
metadata$Nodes <- as.factor(metadata$Nodes)
metadata$Lymphnodes <- as.factor(metadata$Lymphnodes)

summary(metadata)

     SAMPLE_ID    Timepoint                          GROUP
 CNR1001T1:  1   T1:236     NO_RELAPSE                  :96
 CNR1002T1:  1   T2:  0     SYTEMIC_RELAPSE_NO_CNS      :64
 CNR1003T1:  1              CNS_RELAPSE_RCHOP           :39
 CNR1006T1:  1              TESTICULAR_NO_CNS_RELAPSE   :12
 CNR1007T1:  1              CNS_DIAGNOSIS               :11
 CNR1008T1:  1              CNS_RELAPSE_CHOPorEQUIVALENT: 8
 (Other)  :230              (Other)                     : 6
      SITE         Score        Prediction ABClikelihood    Groups
 LN     :127   Min.   :-881   ABC : 92   Min.   :0.00   CNS  : 58
 SO     : 20   1st Qu.: 676   GCB :103   1st Qu.:0.00   CTRL :  6
 TE     : 18   Median :2106   U   : 39   Median :0.02   NOREL:108
 TO     : 16   Mean   :1820   NA's:  2   Mean   :0.47   SYST : 64
 GI     : 11   3rd Qu.:2941              3rd Qu.:1.00
 SP     :  7   Max.   :4323              Max.   :1.00
 (Other): 37   NA's   :2                 NA's   :4
 ABClassify ABCScore  Nodes    Lymphnodes
 ABC:103    ABC: 92   EN: 86   0: 86
 GCB:117    GCB:103   LN:150   1:150
 U  : 16    U  : 41
```

Difference in cases being indexed based on their *cell-of-origin* association subtypes using either of the following features: prediction, ABClassify, ABCScore.

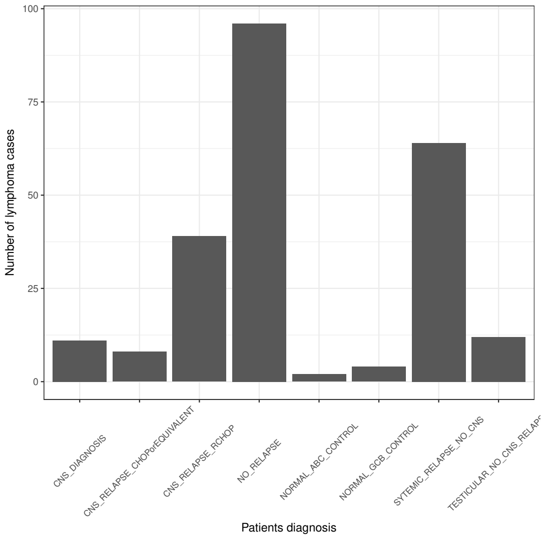
```
metadata %>%
    select(Prediction, ABClassify, ABCScore) %>%
    summary

 Prediction ABClassify ABCScore
 ABC : 92   ABC:103    ABC: 92
 GCB :103   GCB:117    GCB:103
 U   : 39   U  : 16    U  : 41
 NA's:  2
```

Distribution of samples with different treatments.

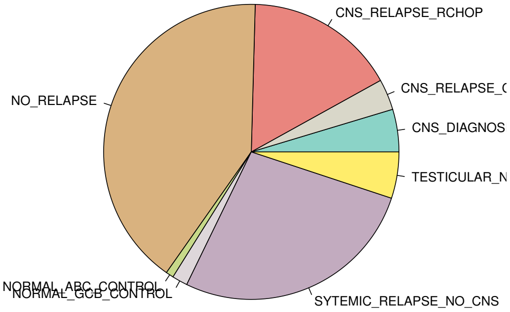
```
metadata %>%
    select(GROUP) %>%
    ggplot(aes(x = GROUP)) +
    geom_histogram(stat = "count") +
    labs(y = "Number of lymphoma cases",
         x = "Patients diagnosis") +
    theme_bw() +
    theme(axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))

Warning:  Ignoring unknown parameters:  binwidth, bins, pad
```



Or as a pie chart.

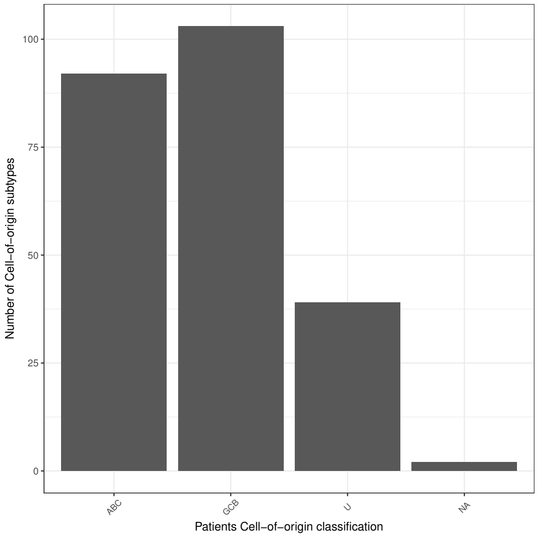
```
palette.pies <- brewer.pal(12, name = "Set3")
palette.pies.adj <- colorRampPalette(palette.pies)(length(unique(metadata$GROUP)))
pie(table(metadata$GROUP), col=palette.pies.adj)
```



Distribution of samples with different cells of origin subtypes.

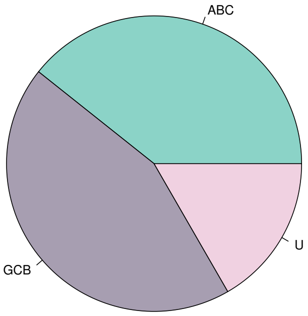
```
metadata %>%
    select(Prediction) %>%
    ggplot(aes(x = Prediction)) +
    geom_histogram(stat = "count") +
    labs(y = "Number of Cell-of-origin subtypes",
         x = "Patients Cell-of-origin classification") +
    theme_bw() +
    theme(axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))

Warning:  Ignoring unknown parameters:  binwidth, bins, pad
```



Or as pie chart.

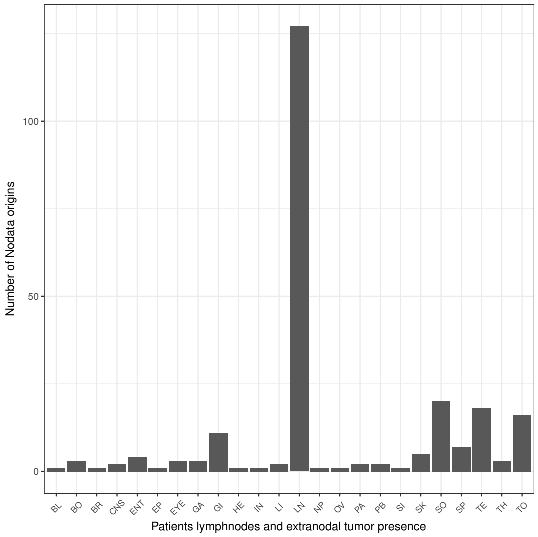
```
palette.pies <- brewer.pal(12, name = "Set3")
palette.pies.adj <- colorRampPalette(palette.pies)(length(unique(metadata$Prediction)))
pie(table(metadata$Prediction), col=palette.pies.adj)
```



Distribution of samples with different lymphnodes and extranodal cancer metastasis.

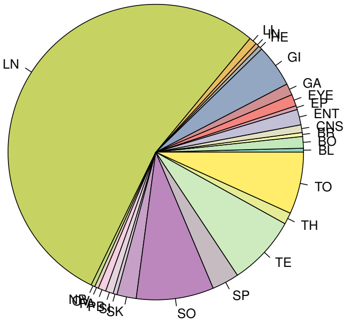
```
par(mfrow=c(2,2))
metadata %>%
    select(SITE) %>%
    ggplot(aes(x = SITE)) +
    geom_histogram(stat = "count") +
    labs(y = "Number of Nodata origins",
         x = "Patients lymphnodes and extranodal tumor presence") +
    theme_bw() +
    theme(axis.text.x = element_text(vjust = .5,
                                     angle = 45,
                                     size = 8))
```

```
Warning:  Ignoring unknown parameters:  binwidth, bins, pad
```



Or as a pie chart.

```
palette.pies <- brewer.pal(12, name = "Set3")
palette.pies.adj <- colorRampPalette(palette.pies)(length(unique(metadata$SITE)))
pie(table(metadata$SITE), col=palette.pies.adj)
```



2 Differential expression

Genes have been fitted in a model that is based on an Empirical Bayes approach. Ranking of the genes determine if they are statistically significant. Bonferroni correction is used to control the false discovery rate (FDR). Moderated t-statistics, FDR, and fold change (log2) are implemented to reduce selection of false positives.

- **adjpval** is the adjusted P-value to control the FDR using Bonferroni correction. **Genes selected here based on their adjpval are also greater than or equal to the bstat threshold**.
- **avgex** is the average expression the ordinary arithmetic average of the log2-expression values for the probe, across all arrays. **Genes selected here based on their avgex are also greater than or equal to the bstat threshold**.
- **bstat** is the moderated t-statistics using an Empirical Bayes approach generating B-statistics scores.

```
expression <- read.table("data/summary.full.90800.txt", sep = "\t", header = T) %>%
    select(Design, Model, Bthreshold, adjPval, Category, Parameter, Transcripts) %>%
    filter(Category == "total")
summary(expression)

                     Design                                Model
 CNSvsNOREL_ABC         : 54   systemicRelapse               : 54
 CNSvsNOREL_GCB         : 54   systemicRelapseCOOclasses     :162
 CNSvsSYST_ABC          : 54   systemicRelapseCOOprediction:162
 CNSvsSYST_GCB          : 54   systemicRelapseCOOscores      :162
 diffCNSvsNOREL_ABCvsGCB: 54   systemicRelapseNodes          :162
 diffCNSvsSYST_ABCvsGCB : 54
 (Other)                :378
   Bthreshold        adjPval          Category      Parameter
 Min.   :-2.00   Min.   :0.049   down :  0    adjpval:234
 1st Qu.:-1.00   1st Qu.:0.049   total:702    avgex  :234
 Median : 0.25   Median :0.049   up   :  0    bval   :234
 Mean   : 0.00   Mean   :0.049
 3rd Qu.: 1.00   3rd Qu.:0.049
 Max.   : 1.50   Max.   :0.049

  Transcripts
 Min.   :    0
 1st Qu.:    2
 Median :   46
 Mean   :  580
 3rd Qu.:  463
 Max.   :10578
```

Number of transcripts when comparing B-statistics scores, which represent confidence in selecting each significantly expressed gene.

```
aggregate( Transcripts ~ Bthreshold, data=expression, FUN=range)

  Bthreshold Transcripts.1 Transcripts.2
1       -2.0             0         10578
2       -1.0             0          6448
3        0.0             0          3618
4        0.5             0          2688
5        1.0             0          1976
6        1.5             0          1429
```

Number of transcripts when samples are classed into groups, which are based on clinical data (e.g., cell-of-origin, CNS relapse, and nodal/extranodal tumor transmission).

```
aggregate( Transcripts ~ Model, data=expression, FUN=range)

                         Model Transcripts.1 Transcripts.2
1               systemicRelapse             0          4938
2     systemicRelapseCOOclasses             0         10578
3 systemicRelapseCOOprediction             0         10578
4      systemicRelapseCOOscores             0         10578
5          systemicRelapseNodes             0          6609
```

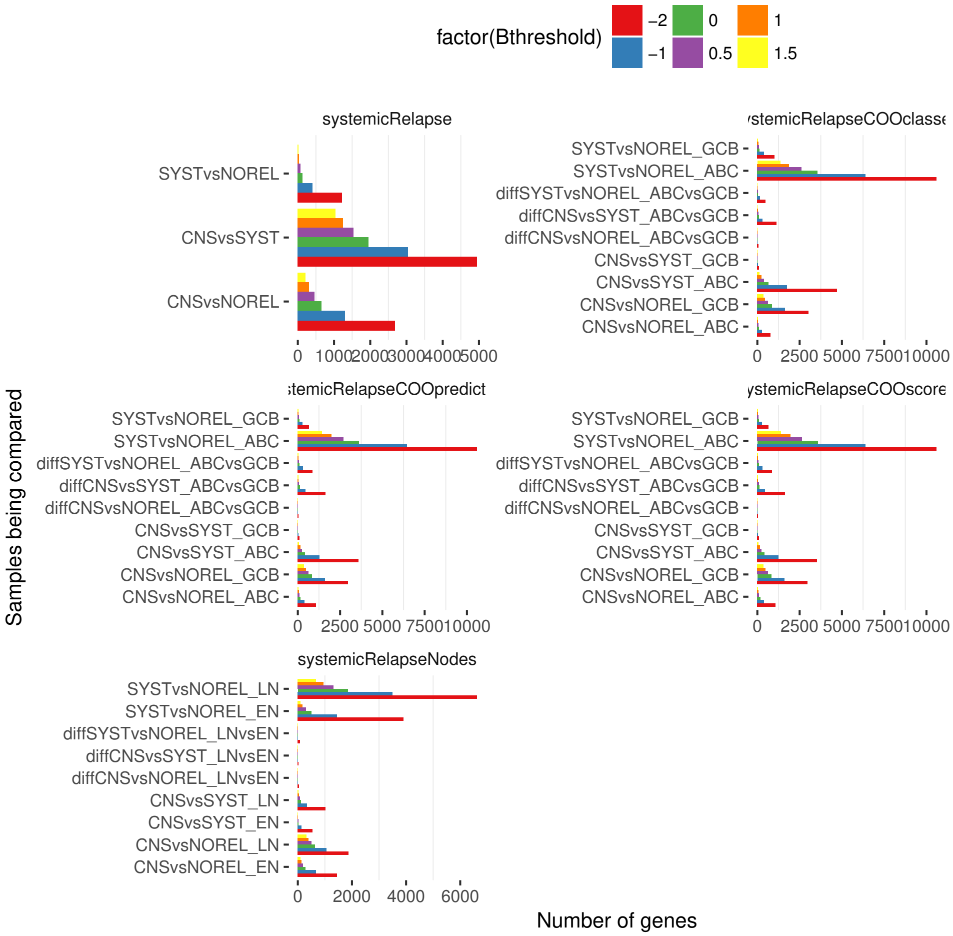
Number of transcripts found when comparing different sample cases indexed based on their clinical data.

```
aggregate( Transcripts ~ Design, data=expression, FUN=range)

                       Design Transcripts.1 Transcripts.2
1                  CNSvsNOREL           116          2678
2              CNSvsNOREL_ABC             2          1082
3               CNSvsNOREL_EN            51          1442
4              CNSvsNOREL_GCB           130          3019
5               CNSvsNOREL_LN           125          1873
6                   CNSvsSYST           441          4938
7               CNSvsSYST_ABC             2          4691
8                CNSvsSYST_EN             3           547
9               CNSvsSYST_GCB             0            98
10               CNSvsSYST_LN             0          1014
11  diffCNSvsNOREL_ABCvsGCB             0            58
12    diffCNSvsNOREL_LNvsEN             0            37
13   diffCNSvsSYST_ABCvsGCB             1          1640
14     diffCNSvsSYST_LNvsEN             0            23
15 diffSYSTvsNOREL_ABCvsGCB             0           868
16   diffSYSTvsNOREL_LNvsEN             0            85
17              SYSTvsNOREL             0          1214
18          SYSTvsNOREL_ABC           704         10578
19           SYSTvsNOREL_EN            35          3907
20          SYSTvsNOREL_GCB             2           994
21           SYSTvsNOREL_LN           295          6609
```

Number of genes that respond to treatment, cell subtypes, and nodal transmission.

```
expression %>%
ggplot(aes(
    x = Design,
    y = Transcripts,
    fill = factor(Bthreshold))) +
theme_bw() +
geom_bar(stat = "identity",
         position = "dodge") +
coord_flip() +
facet_wrap( ~ Model,
          ncol = 2,
           scales = "free") +
scale_fill_brewer(type = "qual", palette = 6) +
labs(x = "Samples being compared",
     y = "Number of genes") +
theme(legend.position = "top",
      strip.background = element_rect(linetype = "blank",
                                      fill = "white"),
      panel.border = element_rect(linetype = "blank",
                                  fill = NA),
      panel.grid.major = element_line(linetype = "blank"))
```



3 Networks

The number of clusters and modules per networks are assigned by designing first a similarity matrix between differentially expressed gene for any two conditions (eg., relapse vs no relapse patient cases). An adjacency matrix is then constructed by weighting the previously inferred measures. The data is transformed to increase the correlation coefficient therefore improving detection of strong correlated patterns. (Example of the strength of data transformation and correlation, visit the following online page).

- **MaxEdgesPerGene**, maximum number of correlations per genes
- **NbNodes**, number of genes found for each edge connection bracket

- **Normalization**, method that focuses on creating complete clusters. We tested methods ranging from Complete clustering, Average, and Ward. Each method is detailed here. Only Complete clustering was retained. All other methods overfitted the data.
- **Correlation**, finding ranges from linear to non-linear trends. We tested Pearson and Spearman correlation.
- **Standardization**, data transformation method. We tested transformation by Hellinger, Standardize, Range, and Logarithmic scaling. Each method is detailed here.
- **MaxGenePerModule**, how many genes assigned by cluster (module)
- **SimilaritySize**, number of initial differentially expressed genes
- **EdgeThreshold**, parameter to limit the weight of the edges
- **CorrelationPower**, power transformation of the data

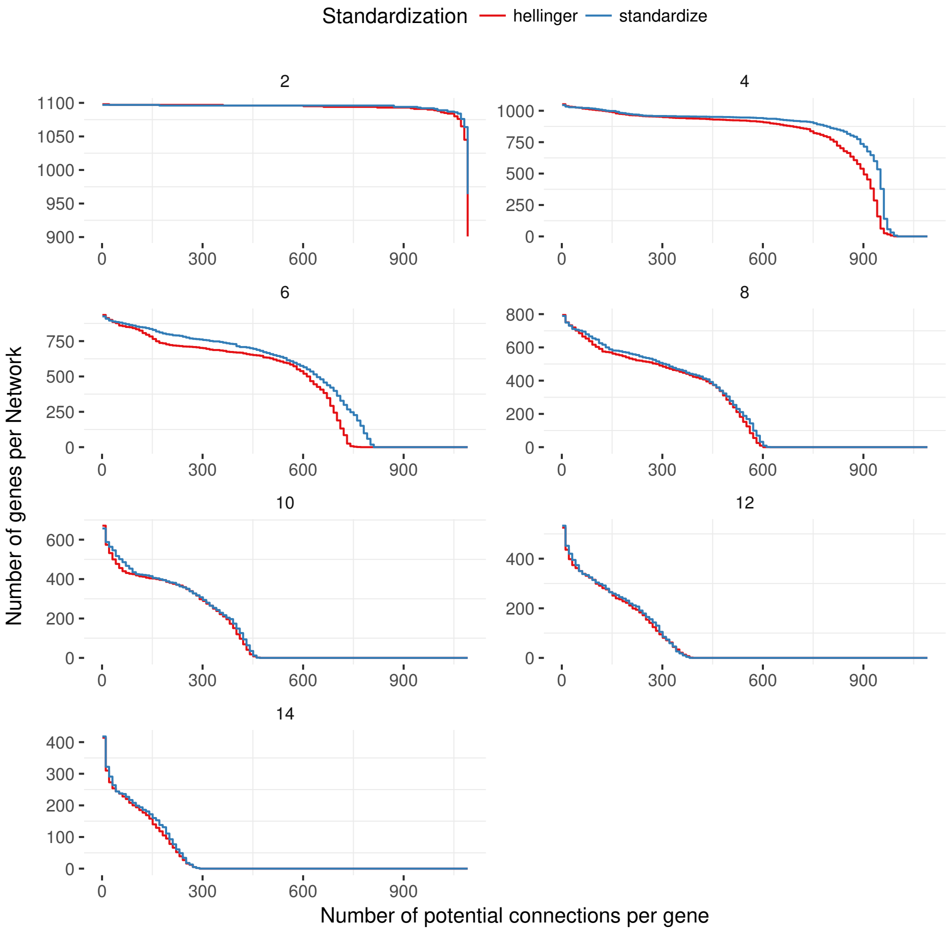
```r
ns <- read.table("./data/networks.summary.104795.txt", header = T)
summary(ns)
```

```
 MaxEdgesPerGene    NbNodes      Normalization    Correlation  
 Min.   :   1    Min.   :   0   complete:4620   spearman:4620  
 1st Qu.: 271    1st Qu.:   0                                  
 Median : 546    Median : 244                                  
 Mean   : 546    Mean   : 406                                  
 3rd Qu.: 821    3rd Qu.: 862                                  
 Max.   :1091    Max.   :1098                                  
    Standardization MaxGenesPerModule SimilaritySize EdgeThreshold
 hellinger  :2310   Min.   :26         Min.   :1099   Min.   :0.5  
 standardize:2310   1st Qu.:36         1st Qu.:1099   1st Qu.:0.5  
                    Median :55         Median :1099   Median :0.5  
                    Mean   :57         Mean   :1099   Mean   :0.5  
                    3rd Qu.:79         3rd Qu.:1099   3rd Qu.:0.5  
                    Max.   :91         Max.   :1099   Max.   :0.5  
 CorrelationPower
 Min.   : 2      
 1st Qu.: 4      
 Median : 8      
 Mean   : 8      
 3rd Qu.:12      
 Max.   :14      
```

Difference between methods used for network inference. Are we able to generate convergence of the output of all iterations across all methods?

↰Test graphs

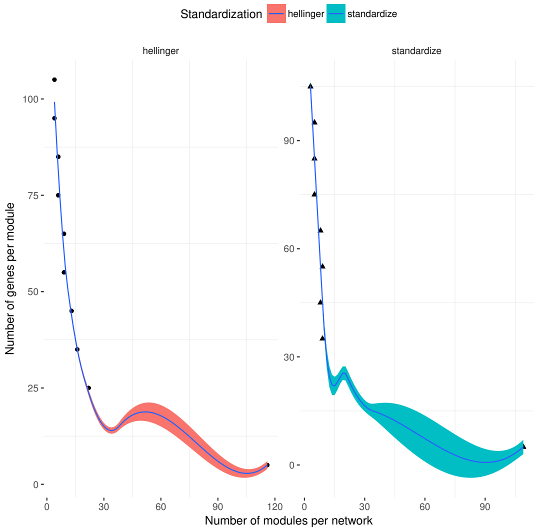
```r
ns %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module. Each module contains differing number of nodes based on their correlation strength. Each cluster contains at least one module. Each network contains at least one cluster. One module can be assigned to nodes that belong to more than one cluster. The Lowess curves show if the trend in the data is linear or not. The wave around Lowess curves represents the level of confidence of the data points (the narrower the interval the better, less variability = more accuracy).

↰Points=iterations. With less iterations comes high variability of the curve

```
read.table("./data/modules.summary.104795.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
                ncol = 2,
                scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                           fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.1 Network analysis for Spearman-related correlations

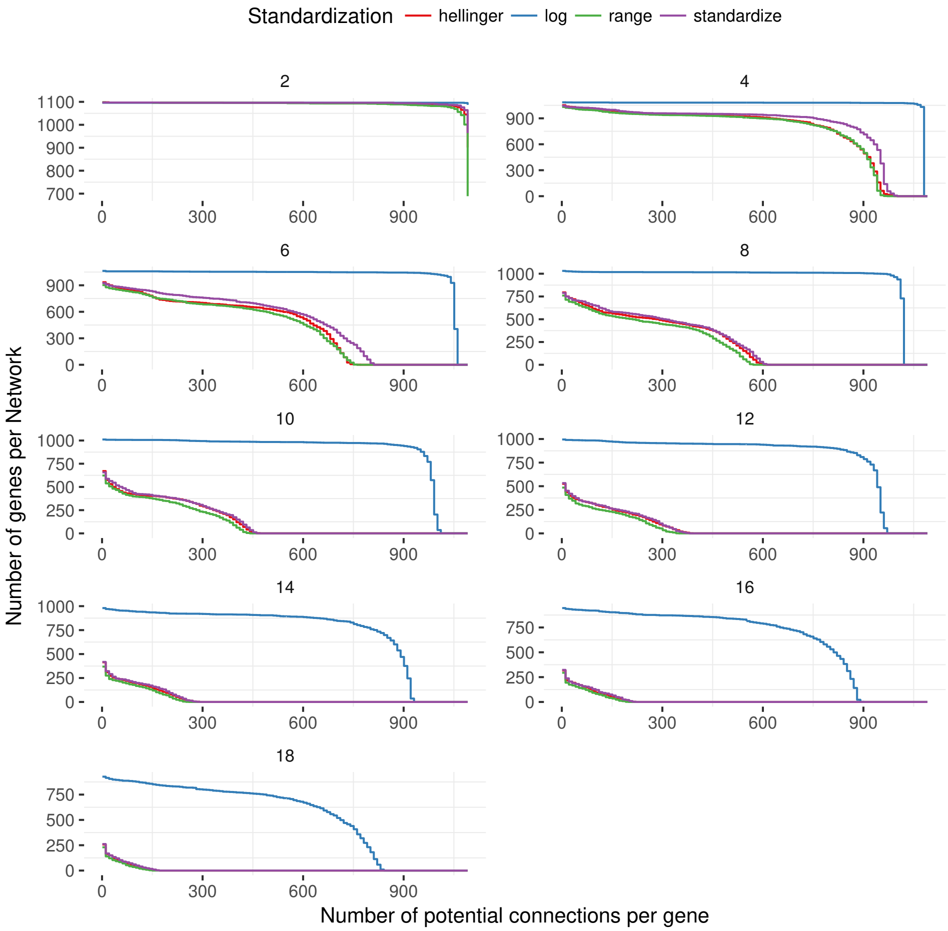
Thresholds based on the Empirical Bayes approach to rank genes and determine if a gene is significantly expressed. Limma implementation.

- **Average Expression**: 5
- **Adjusted P-value**: equal or less than 0.045
- **Log Fold Change**: 1
- **B-statisitcs**: 1.5

3.1.1 Nodal versus extra-nodal lymphoma

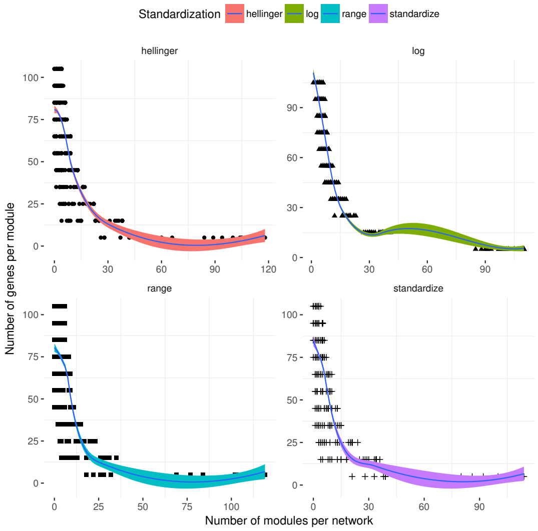
Genetic networks from differentially expressed genes selected by comparing sample cases with nodal and extranodal lymphoma.

```
read.table("./data/networks.summary.104859.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

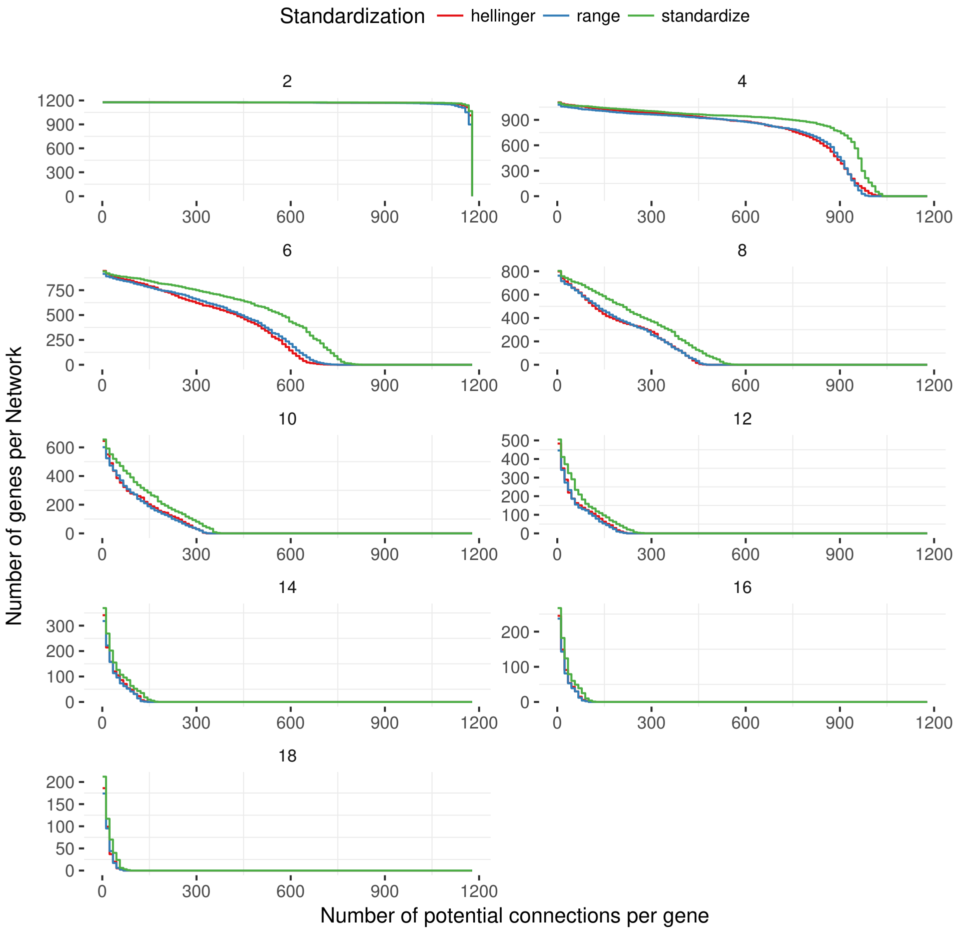
```
read.table("./data/modules.summary.104859.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.1.2 Relapsed versus no CNS relapsed cases

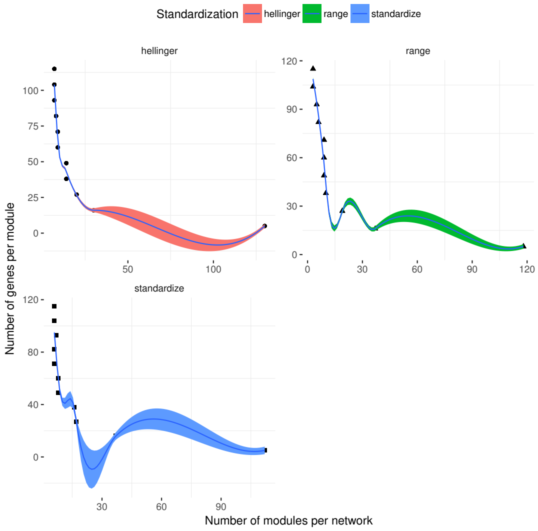
Genetic networks from differentially expressed genes selected by comparing sample cases with systemic or no CNS relapse lymphoma.

```
read.table("./data/networks.summary.114018.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

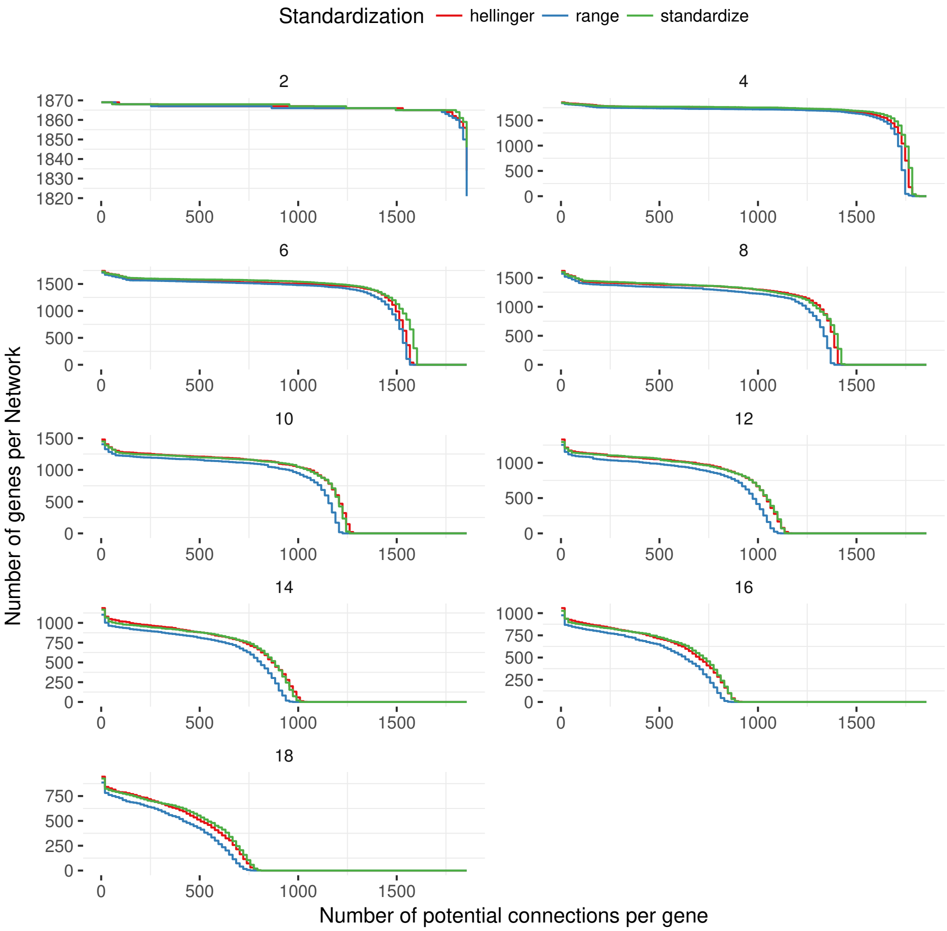
```
read.table("./data/modules.summary.114018.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.1.3 Lymphoma cases classified by Cell-of-origin subtypes

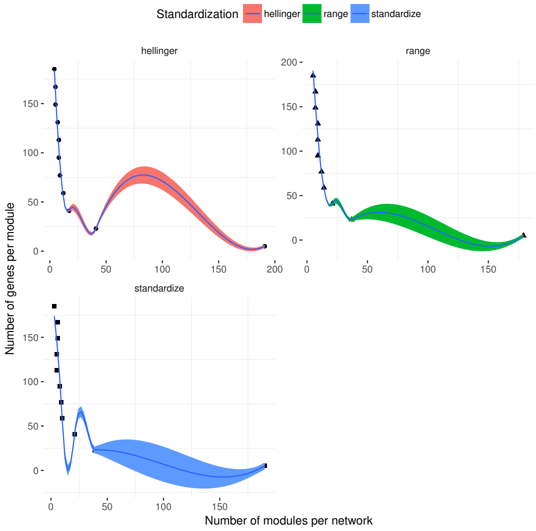
Genetic networks from differentially expressed genes selected by comparing sample cases with systemic or no CNS relapse lymphoma.

```r
read.table("./data/networks.summary.114017.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

```
read.table("./data/modules.summary.114017.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.2 Network analysis for Pearson-related correlations

Thresholds based on the Empirical Bayes approach to rank genes and determine if a gene is significantly expressed. Limma implementation.

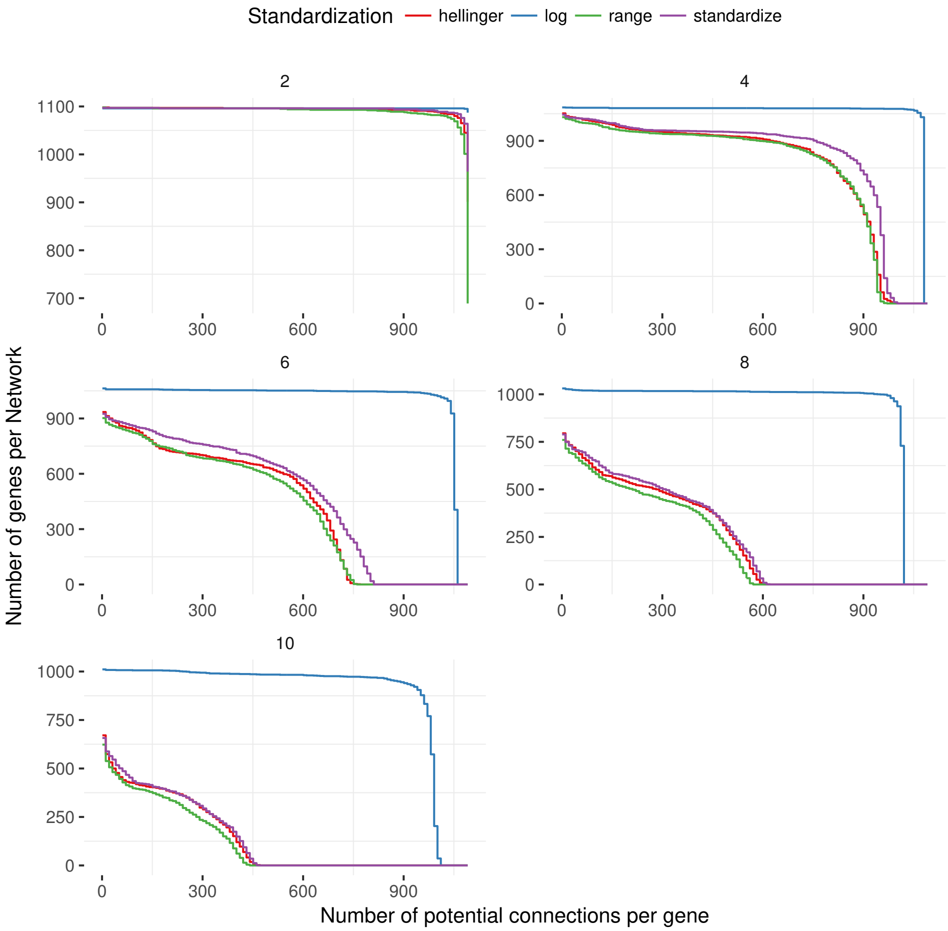
↰With pearson, we can only raise the data to power 10. All are discarded after 10.

- **Average Expression**: 5
- **Adjusted P-value**: equal or less than 0.045
- **Log Fold Change**: 1
- **B-statisitcs**: 1.5

3.2.1 Nodal versus extra-nodal lymphoma

Genetic networks from differentially expressed genes selected by comparing sample cases with nodal and extranodal lymphoma.

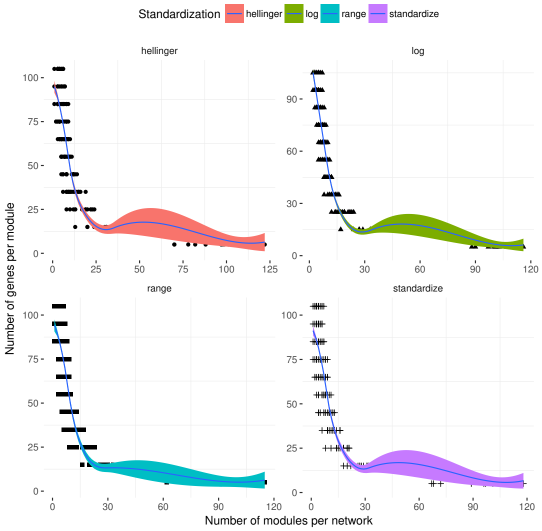
```r
read.table("./data/networks.summary.104862.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

↰Since Lowess ranks by confidence, Log transformation seems the best, ie, low variability. For this, Log is removed from further tests.

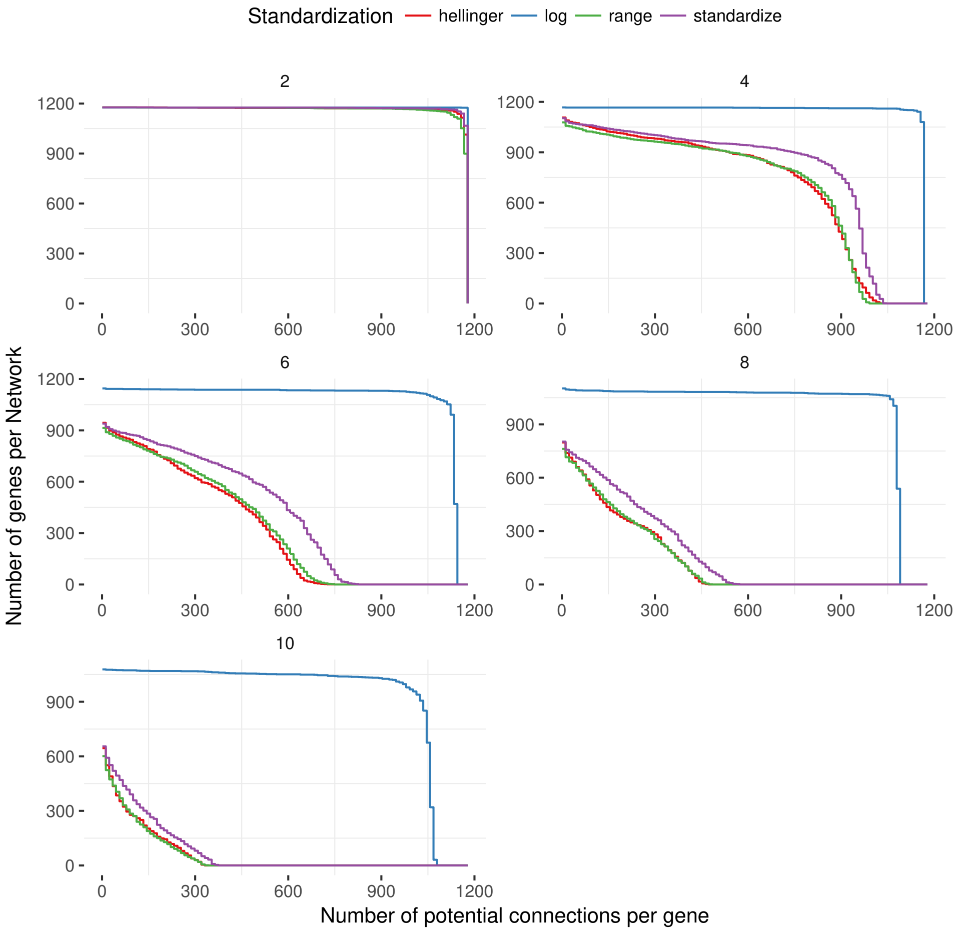
```
read.table("./data/modules.summary.104862.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
                ncol = 2,
                scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.2.2 Relapsed versus no CNS relapsed cases

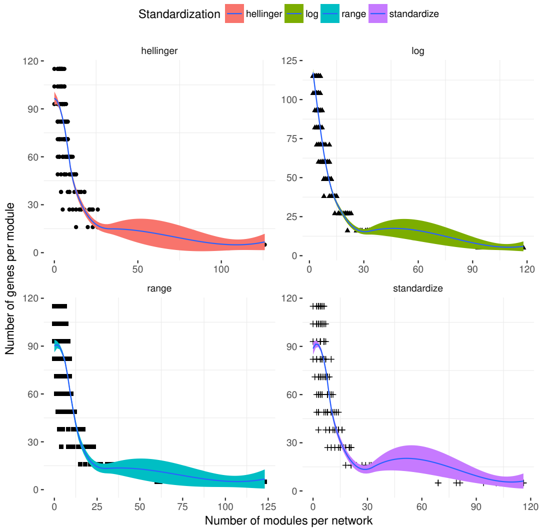
Genetic networks from differentially expressed genes selected by comparing sample cases with systemic or no CNS relapse lymphoma.

```
read.table("./data/networks.summary.104863.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

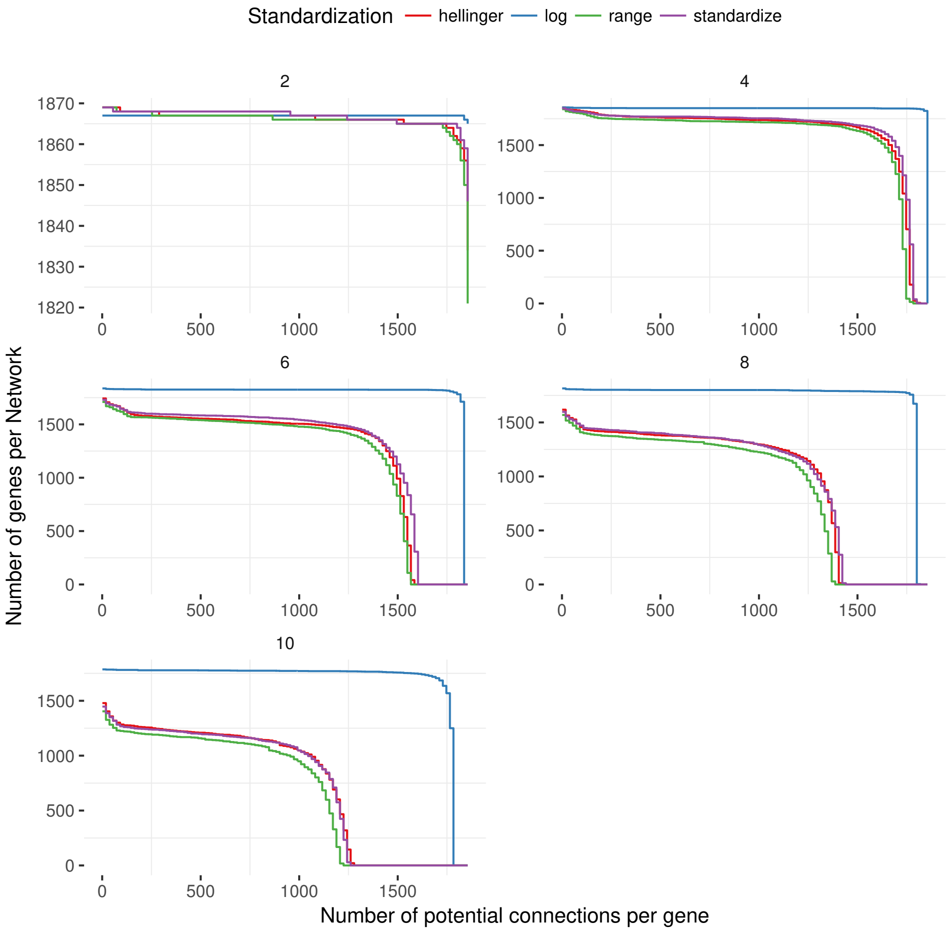
```
read.table("./data/modules.summary.104863.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.2.3 Lymphoma cases classified by Cell-of-origin subtypes

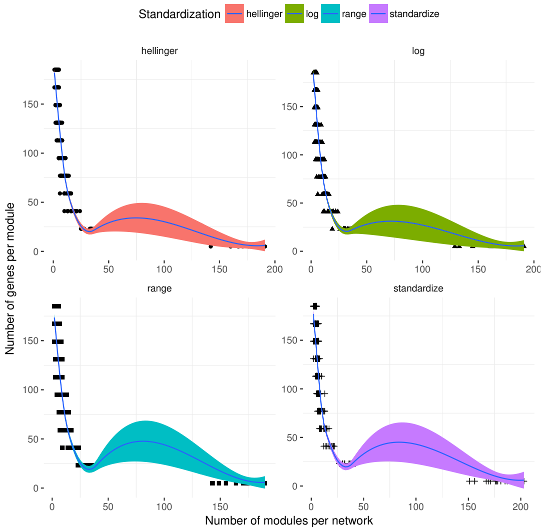
Genetic networks from differentially expressed genes selected by comparing sample cases with cell of origin classification based on ABC or GCB subtypes.

```
read.table("./data/networks.summary.104864.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

```
read.table("./data/modules.summary.104864.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.3 Network analysis for Spearman-related correlations

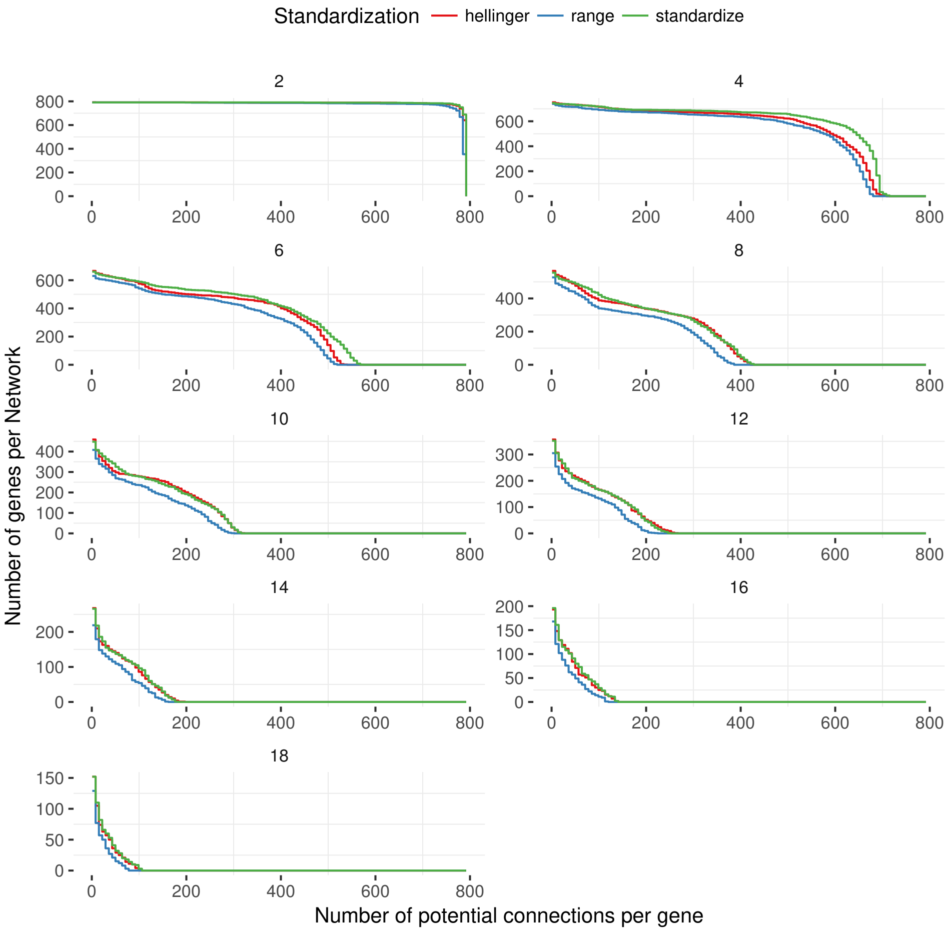
Thresholds based on the Empirical Bayes approach to rank genes and determine if a gene is significantly expressed. Limma implementation.

- **Average Expression**: 10
- **Adjusted P-value**: equal or less than 0.030
- **Log Fold Change**: 1
- **B-statisitcs**: 2

3.3.1 Nodal versus extra-nodal lymphoma

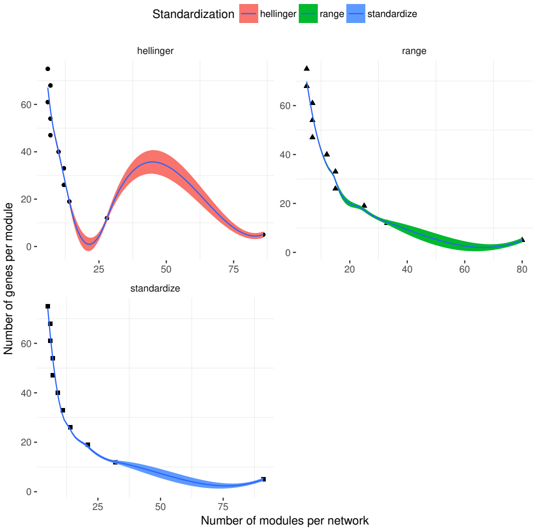
Genetic networks from differentially expressed genes selected by comparing sample cases with nodal and extranodal lymphoma.

```
read.table("./data/networks.summary.119759.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

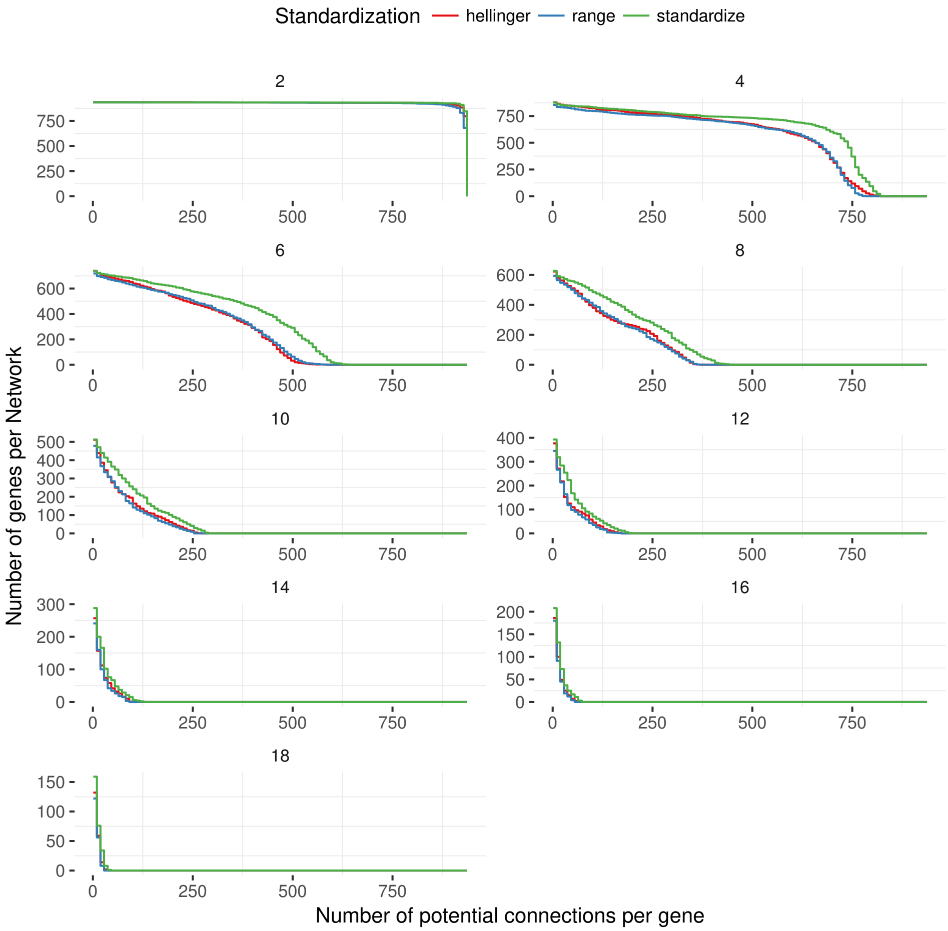
```r
read.table("./data/modules.summary.119759.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.3.2 Relapsed versus no CNS relapsed cases

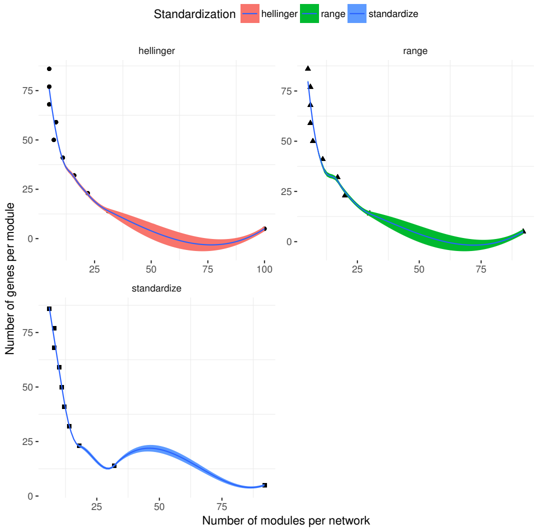
Genetic networks from differentially expressed genes selected by comparing sample cases with systemic or no CNS relapse lymphoma.

```
read.table("./data/networks.summary.119760.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

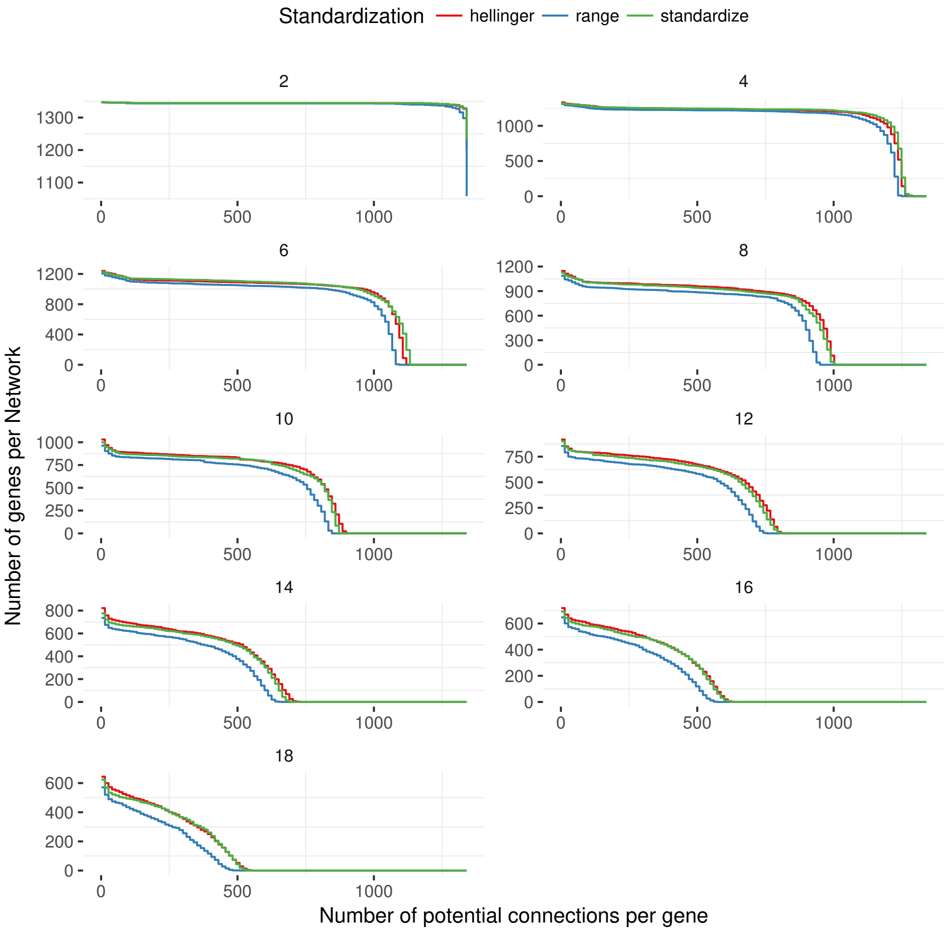
```
read.table("./data/modules.summary.119760.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.3.3 Lymphoma cases classified by Cell-of-origin subtypes

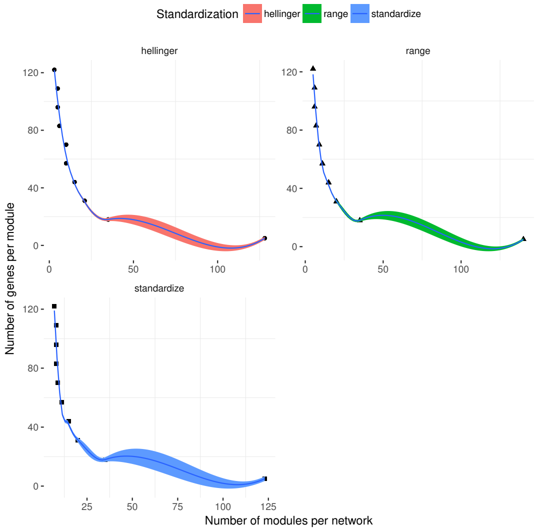
Genetic networks from differentially expressed genes selected by comparing sample cases with systemic or no CNS relapse lymphoma.

```
read.table("./data/networks.summary.119758.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

```r
read.table("./data/modules.summary.119758.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.4 Network analysis for Pearson-related correlations

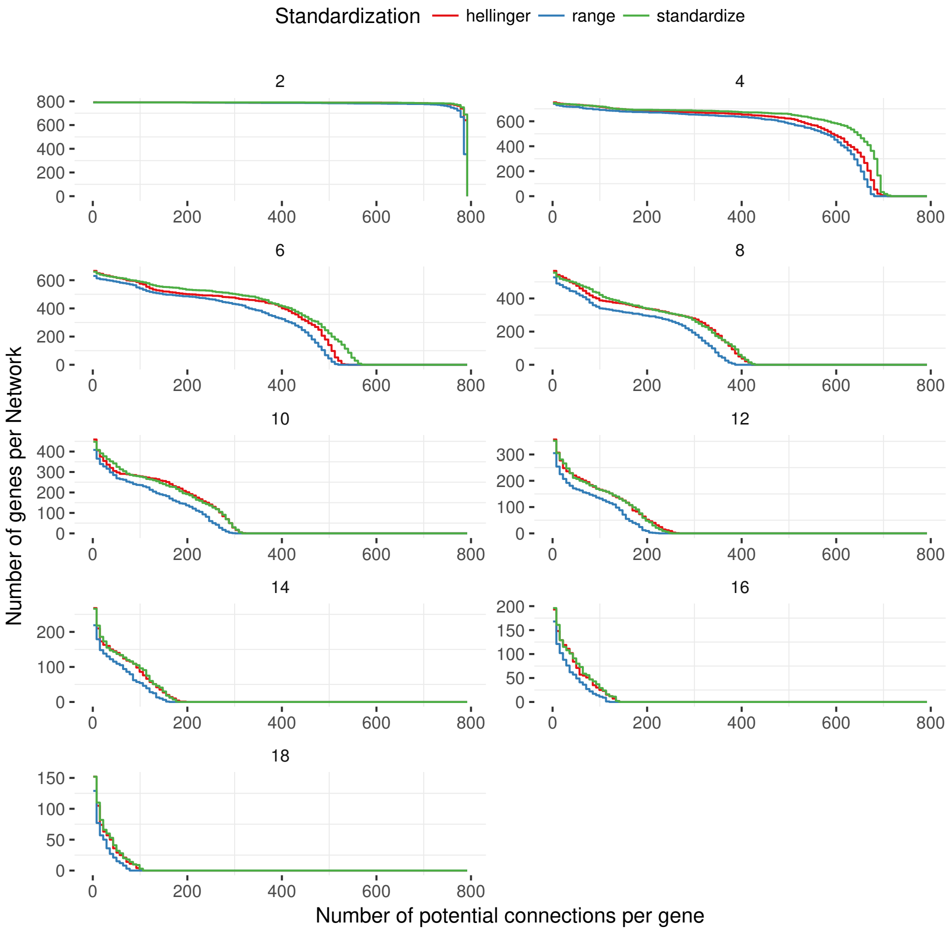
Thresholds based on the Empirical Bayes approach to rank genes and determine if a gene is significantly expressed. Limma implementation.

- **Average Expression**: 10
- **Adjusted P-value**: equal or less than 0.030
- **Log Fold Change**: 1
- **B-statisitcs**: 2

3.4.1 Nodal versus extra-nodal lymphoma

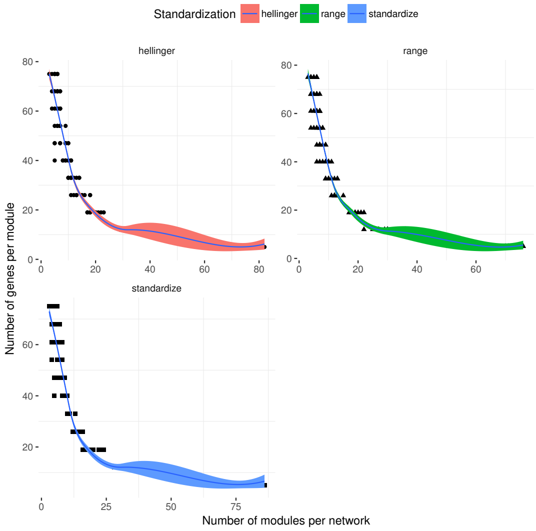
Genetic networks from differentially expressed genes selected by comparing sample cases with nodal and extranodal lymphoma.

```r
read.table("./data/networks.summary.119755.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

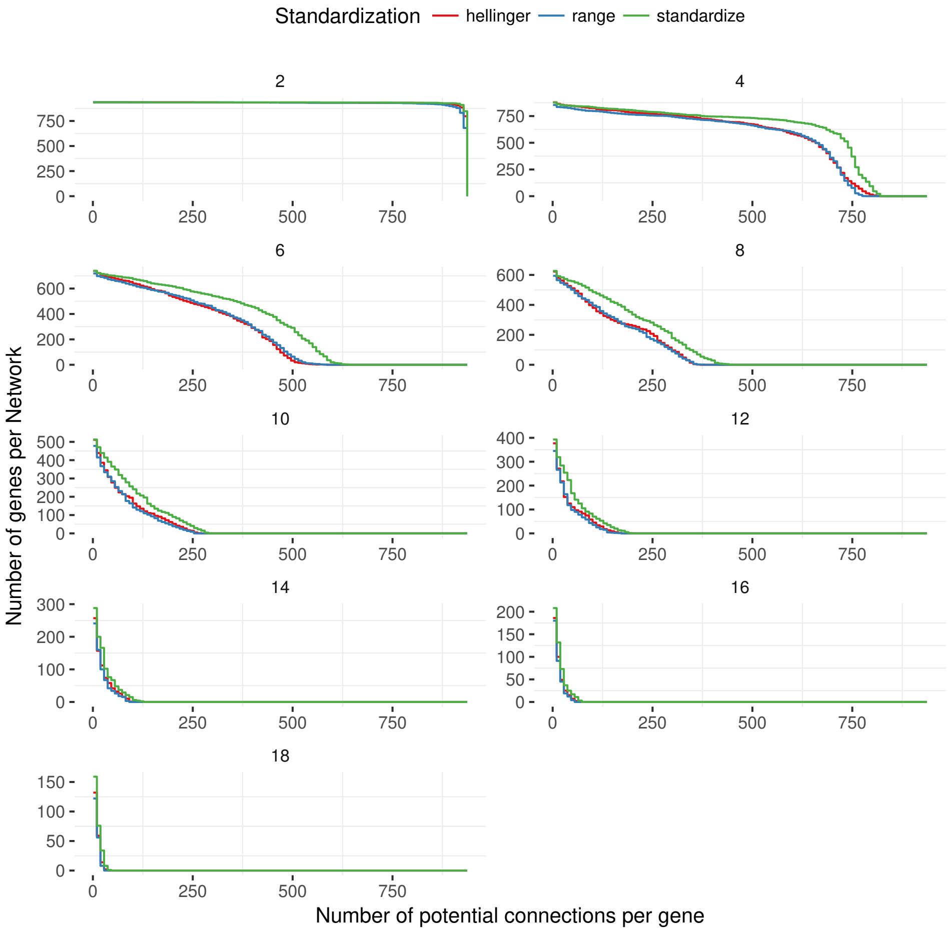
```
read.table("./data/modules.summary.119755.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                           fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.4.2 Relapsed versus no CNS relapsed cases

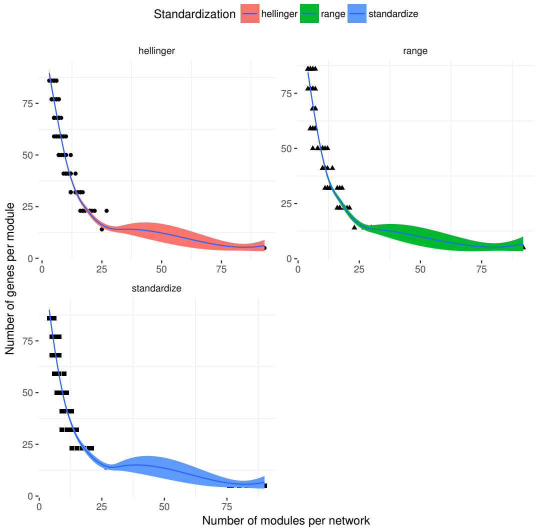
Genetic networks from differentially expressed genes selected by comparing sample cases with systemic or no CNS relapse lymphoma.

```
read.table("./data/networks.summary.119754.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

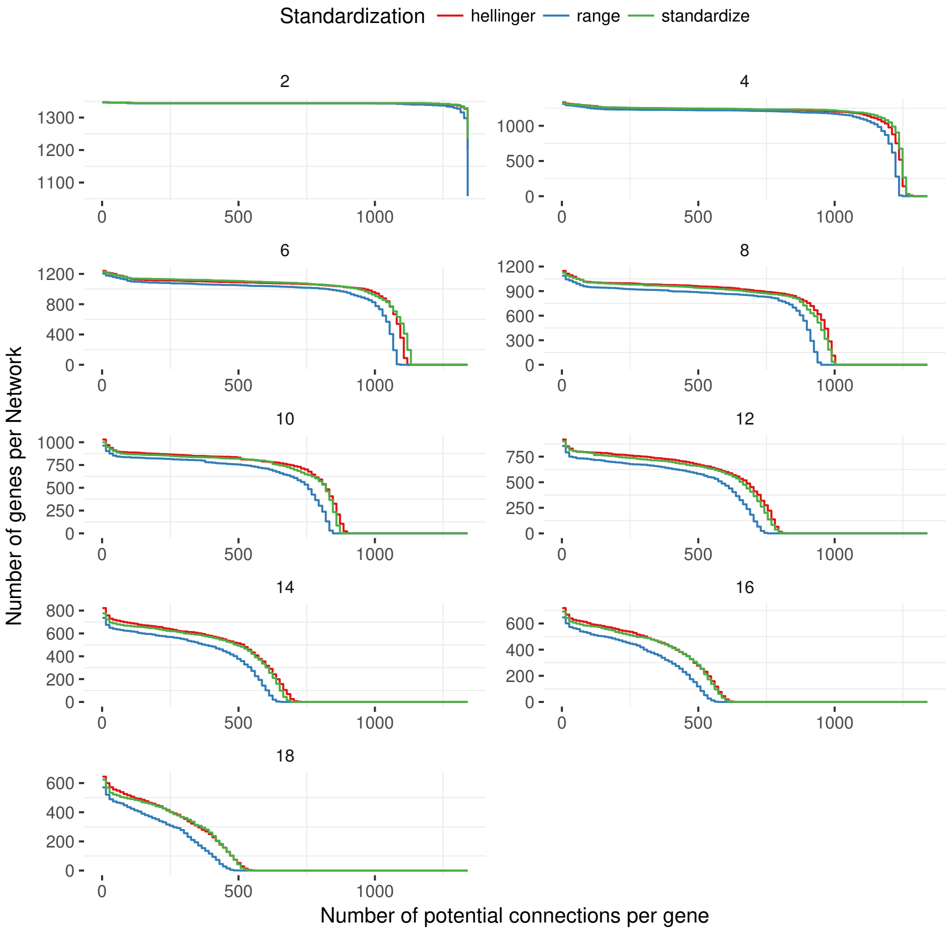
```
read.table("./data/modules.summary.119754.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



3.4.3 Lymphoma cases classified by Cell-of-origin subtypes

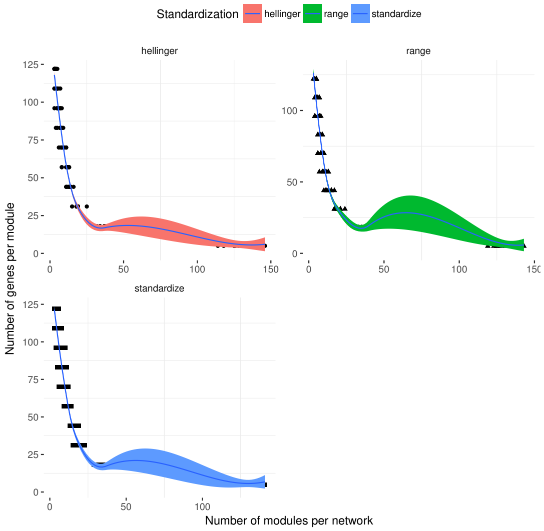
Genetic networks from differentially expressed genes selected by comparing sample cases with cell of origin classification based on ABC or GCB subtypes.

```
read.table("./data/networks.summary.119757.txt", header = TRUE) %>%
    ggplot(aes(
        x = MaxEdgesPerGene,
        y = NbNodes,
        fill = Standardization)) +
    theme_bw() +
    geom_step(aes(color = Standardization),
              stat = "identity") +
    facet_wrap( ~ CorrelationPower,
               ncol = 2,
               scales = "free") +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of potential connections per gene",
         y = "Number of genes per Network") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank"))
```



Showing the number of modules per network and the number of genes per module.

```
read.table("./data/modules.summary.119757.txt", header = TRUE) %>%
    ggplot(aes(
        x = NbModules,
        y = MaxGenesPerModule,
        fill = Standardization)) +
    theme_bw() +
    geom_point(aes(shape = Standardization)) +
    scale_color_brewer(type = "qual", palette = 6) +
    labs(x = "Number of modules per network",
         y = "Number of genes per module") +
    facet_wrap( ~ Standardization,
               ncol = 2,
               scales = "free") +
    theme(legend.position = "top",
          strip.background = element_rect(linetype = "blank",
                                          fill = "white"),
          panel.border = element_rect(linetype = "blank",
                                      fill = NA),
          panel.grid.major = element_line(linetype = "blank")) +
    geom_smooth(method = 'loess', size = .5, level = 0.5, alpha=1)
```



4 System Information

The version number of R and packages loaded for generating the vignette were:

```
###save(list=ls(pattern=".*|.*"),file="PD.Rdata")
sessionInfo()

R version 3.4.3 (2017-11-30)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: elementary OS 0.4.1 Loki

Matrix products: default
BLAS: /usr/lib/libblas/libblas.so.3.6.0
LAPACK: /usr/lib/lapack/liblapack.so.3.6.0

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

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

other attached packages:
 [1] bindrcpp_0.2        DescTools_0.99.23   igraph_1.1.2
 [4] tidyr_0.7.2         dplyr_0.7.4         ggplot2_2.2.1
 [7] latticeExtra_0.6-28 RColorBrewer_1.1-2  lattice_0.20-35
[10] leaps_3.0           gdata_2.18.0        knitr_1.17

loaded via a namespace (and not attached):
 [1] Rcpp_0.12.13     pillar_1.1.0     compiler_3.4.3
 [4] plyr_1.8.4       highr_0.6        bindr_0.1
 [7] tools_3.4.3      digest_0.6.12    boot_1.3-20
[10] evaluate_0.10.1  tibble_1.4.2     manipulate_1.0.1
[13] gtable_0.2.0     pkgconfig_2.0.1  rlang_0.1.2
[16] Matrix_1.2-11    expm_0.999-2     mvtnorm_1.0-6
[19] stringr_1.2.0    gtools_3.5.0     grid_3.4.3
[22] glue_1.2.0       R6_2.2.2         foreign_0.8-69
[25] purrr_0.2.4      magrittr_1.5     scales_0.5.0
[28] MASS_7.3-47      assertthat_0.2.0 colorspace_1.3-2
[31] labeling_0.3     stringi_1.1.5    lazyeval_0.2.1
[34] munsell_0.4.3
```