GSReg: A Package for Gene Set Variability Analysis

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1 Introduction

The `GSReg` package allows to analyze pathways based on the variability of the expression of sets of genes that are targets of those pathways. Basing this set statistic on variability enables inference of dysregulated pathways in diseases, including notably cancers. The first set statistic for gene variability was in the work of Eddy and his colleagues (see [1]) which used a ranked based methodology called *DIRAC*. *DIRAC* calculates a measure of variability of the ordering of the expression of genes in a pathway for specific phenotype. The basic idea

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behind *DIRAC* is to generate a template for the pair-wise comparisons of gene expressions of a pathway within a phenotype. DIRAC calculates a measure of the variability of the ordering within the phenotype, i.e. the expected distance of a sample from the phenotype and the template of the phenotype. In mathematical terms, if we denote denote two i.i.d. samples from the same phenotypes by X and X' and D Kendall-τ-distance on the specific pathways, then the EVA [**?**] statistic is $E(D(X, X'))$. It identifies significantly dysregulated pathways by estimating p-values from a permutation test. Eddy et al. found that more pathological phenotypes usually have more pathways with higher variability compared to less pathological phenotypes.

However, the permutation test in DIRAC is computationally intensive and reaching low p-values may be impractical since they require a huge number of permutations. Low p-values are required for multiple hypothesis correction. A similar measure of variability of the orderings of gene sets was proposed in [2]. This method approximates the p-value theoretically, without a permutation test. This method is based on Kendall-τ distance [3] and the theory of U-Statistics, thus we call this method Gene Set Expression Variation Analysis (or in short EVA). Specifically, Kendall-τ distance between two expression profiles counts the number of disagreeing pairwise comparisons between two profiles. The EVA measures the variability of the gene expression of pathway genes from a phenotype by calculating the expectation of Kendall-τ distance between two random samples from the phenotype. EVA then identifies if the variability is significantly different across two phenotypes. To approximate this p-value EVA applies a U-Statistic Theory approach.

The `GReg` package contains two following utilities:

1. Identifying the dysregulated pathways with *DIRAC* measure of variability. The significance is calculated using permutation test. This is the first time that DIRAC analysis has been implemented in R. It also is more adaptable to new datasets than the original Matlab code in [1].

2. Identifying the dysregulated pathways with *EVA* measure of variability. The significance is approximated through applying U-statistics theory. This is very time efficient and consistent with both *DIRAC* and applying permutation test on EVA.

2 Input Data

2.1 Data structure

In short, the `GSReg` package requires the following data in the following format:

1. Gene Expression Data

   (a) The expression be in the form of a matrix where rows represent genes (or probes) and columns represent samples.

   (b) The expression matrix cannot have NAs.

   (c) The expression matrix rows must have names of genes or the probes.

2. Pathways

   (a) The list of pathways must contain character vectors. Only the elements of the vectors which appear in rownames of the expression matrix are considered for analysis.

   (b) The list of the pathways must have names for each vectors.

3. Phenotypes

   (a) A factor with binary levels.

We used the data provided in the `GSBenchMark` package to reproduce the results in Eddy et al. [1]. The `GSBenchMark` contains data for the pathways as well as the gene expression and phenotype data from twelve studies. We load the information about the pathways from

`GSBenchMark`:

```
> library(GSBenchMark)
> data(diracpathways)
> class(diracpathways)

[1] "list"

> names(diracpathways)[1:5]

[1] "DEATHPATHWAY"       "TCAPOPTOSISPATHWAY" "CCR3PATHWAY"
[4] "NEUTROPHILPATHWAY"  "ALTERNATIVEPATHWAY"

> class(diracpathways[[1]])

[1] "character"
```

AS mentioned `GSReg` package requires the information of the pathways to be as a list of character vectors. Also, `GSReg` requires the pathways to have names. The variable `diracpathways` contains gene pathways. It is a list. Each element represents a pathway with its name. Each elements contains a list of characters which represent the genes in the pathway. e.g. `diracpathways[["DEATHPATHWAY"]]`.

Now, we load the datasets' names:

```
> data(GSBenchMarkDatasets)
> print(GSBenchMark.Dataset.names)

 [1] "leukemia_GSEA"        "marfan_GDS2960"       "melanoma_GDS2735"
 [4] "parkinsons_GDS2519"   "prostate_GDS2545_m_nf" "prostate_GDS2545_m_p"
 [7] "prostate_GDS2545_p_nf" "sarcoma_data"         "squamous_GDS2520"
[10] "breast_GDS807"        "bipolar_GDS2190"
```

The remaining examples in this vignette rely on one of the datasets, i.e. "squamous GDS2520."
Similar analyses may be reproduces for other datasets by selecting a different element of "GS-BenchMark.Dataset.names."

```
> DataSetStudy = GSBenchMark.Dataset.names[[9]]
> print(DataSetStudy)

[1] "squamous_GDS2520"

> data(list=DataSetStudy)
```

The data consists of two variables: `exprsdata` and `phenotypes`. `exprsdata` consists of a gene expression matrix where the rows and columns represent genes and the samples respectively. `GSReg` requires the rownames of gene expression variable represent the gene names, *i.e.* they are represented in the pathway information variable.

The `GSReg` does not allow any missing data. To comply with the requirements we remove genes with NAs. The user may use any imputation to resolve this issue:

```
> if(sum(apply(is.nan(exprsdata),1,sum)>0))
    exprsdata = exprsdata[-which(apply(is.nan(exprsdata),1,sum)>0),];
```

One can extract the gene names by:

```
> genenames = rownames(exprsdata);
> genenames[1:10]

 [1] "MAPK3"  "TIE1"   "CYP2C19" "CXCR5"  "CXCR5"  "DUSP1"  "MMP10"  "DDR1"
 [9] "EIF2AK2" "HINT1"
```

3 Analysis of the pathways

Here, we demonstrate how to use the GSReg package to compute DIRAC and EVA statistics.

3.1 DIRAC Analysis

First, we load the library:

```
> library(GSReg)
```

The package also implements the alternative EVA statistic in the function `GSReg.GeneSets.DIRAC`. This function receives gene expression as `geneexpres`, the pathway information as `pathways` and phenotypes of samples as a factor with two levels and length equal to column number of `geneexpres`. *DIRAC* uses can use either a permutation test or normal approximation for p-value calculation; so, `GSReg.GeneSets.DIRAC` receives the number of permutations through (`Nperm`) with default value equal to 0 which indicates the normal approximation.

```
> Nperm = 10
> system.time({DIRACperm =GSReg.GeneSets.DIRAC(exprsdata,diracpathways,phenotypes,Nperm=Nperm)})

   user  system elapsed
  5.820   0.008   5.831

> system.time({DIRACAn =GSReg.GeneSets.DIRAC(exprsdata,diracpathways,phenotypes)})

   user  system elapsed
  0.488   0.008   0.498

>
```

Here is the histogram of the DIRAC p-values:

```
> hist(DIRACAn$pvalues,xlab="pvalue",main="Hist of pvalues applying DIRAC Analysis.")
```

Hist of pvalues applying DIRAC Analysis.
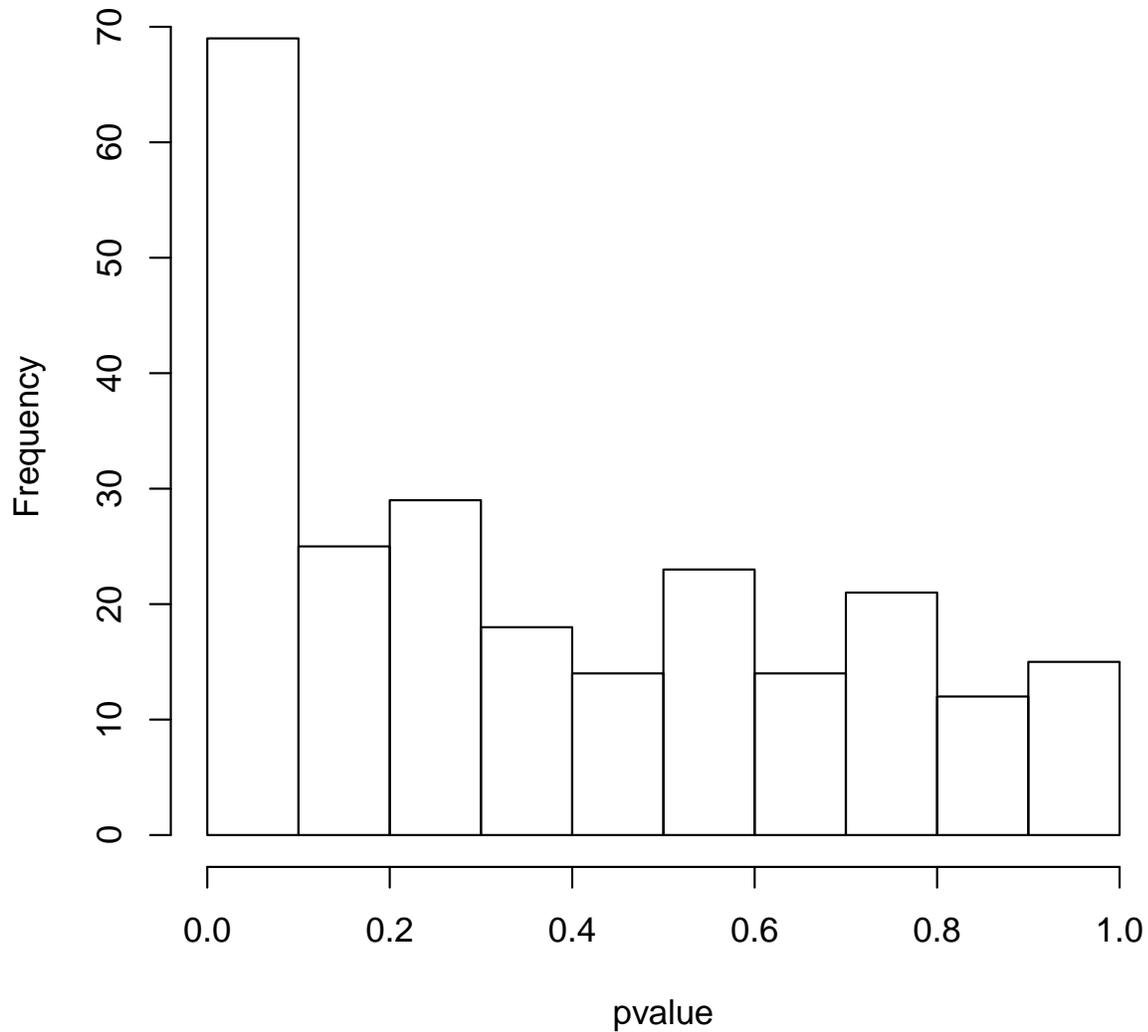
To check if the approximations are reliable, we plot the z-scores calculated to approximate p-values versus the p-values from the permutation tests.

Figure 2 shows the result of comparing p-value DIRAC computing from 1000 permutation test and approximation using normal approximation (offline generated).

3.2   EVA

The package also implements the alternative EVA statistic in the function `GSReg.GeneSets.EVA`. The function requires the similar inputs as `GSReg.GeneSets.DIRAC` (i.e. `geneexpres`, `path-`

```
> plot(x=abs(DIRACAn$zscores),y=DIRACperm$pvalues,xlab="|Z-score|",
      ylab="p-value",col="red1",main="DIRAC p-value comparisons")
> zscorelin <- seq(min(abs(DIRACAn$zscores)),max(abs(DIRACAn$zscores)),by = 0.1)
> pvaltheoretic = (1-pnorm(zscorelin))*2
> lines(x=zscorelin,y=pvaltheoretic,type="l",pch=50,lty=5,col="darkblue")
> legend("topright",legend=c("permutation test","Normal Approx."),
      col=c("red1","blue"),text.col=c("red1","blue"),
      lty=c(NA,1),lwd=c(NA,2.5),pch=c(21,NA))
```
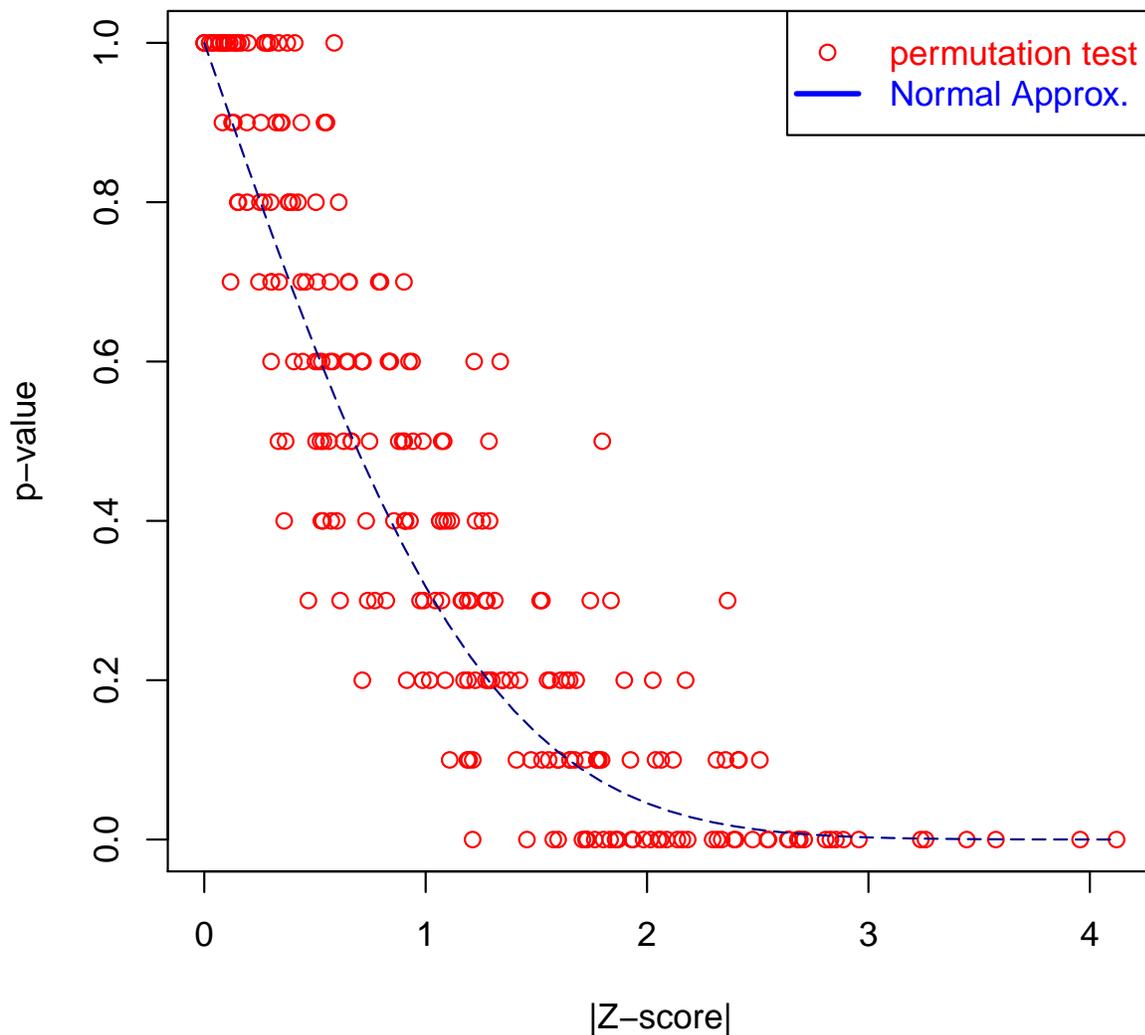

Figure 1: Comparing p-value from permutation test and normal approximation with only 10 permutations.

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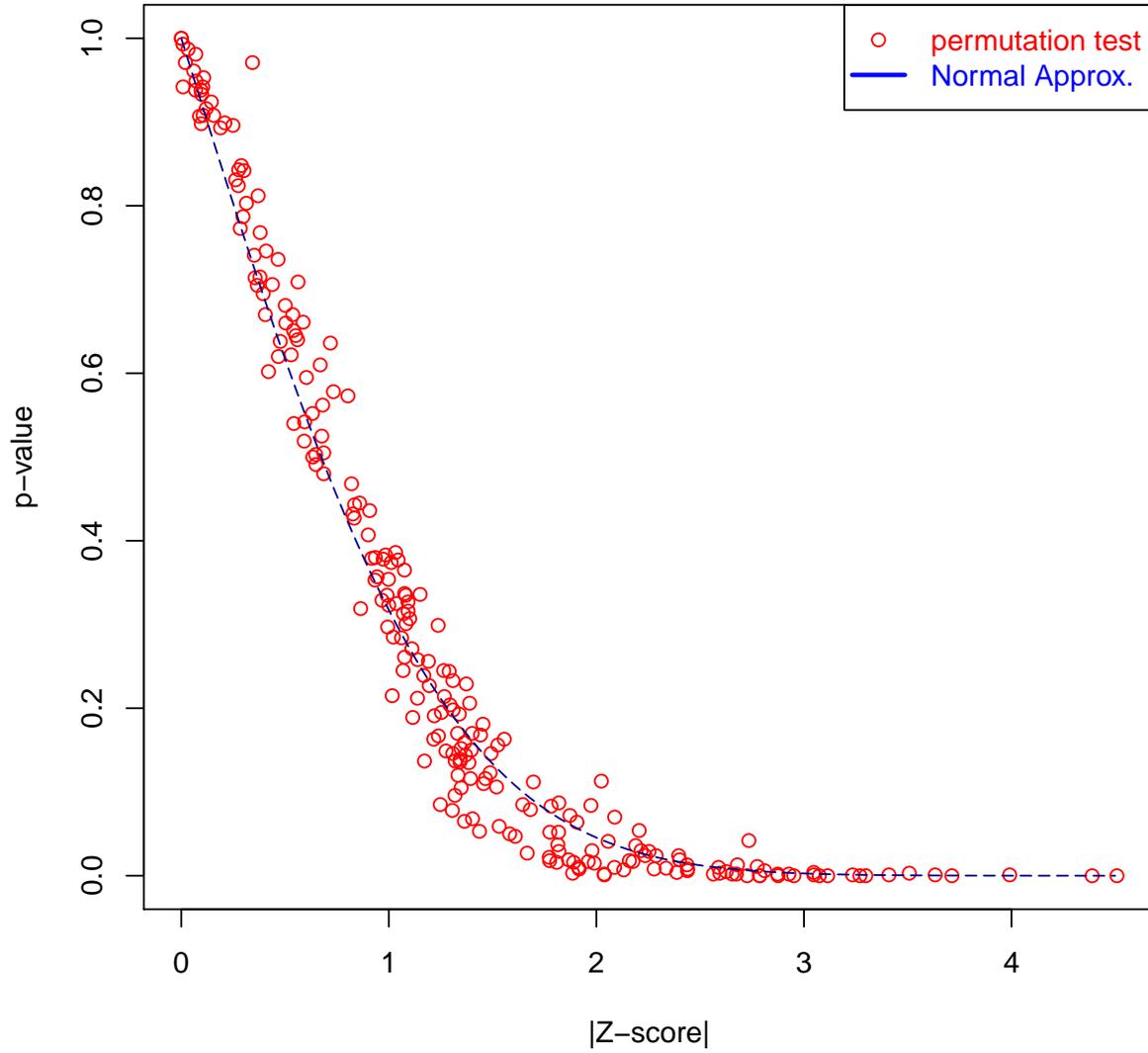
Figure 2: Theoretical p-value versus empirical p-value using 1000 permutations.

ways, `phenotypes`) except it does not need `Nperm` since the p-value is not calculated through permutation test but through the mentioned U-statistic theory approach.

```
> #Calculating the variance for the pathways
> #Calculate how much it takes to calculate the statistics and their p-value for all pathways
>
> system.time({VarAnKendallV = GSReg.GeneSets.EVA(geneexpres=exprsdata,
   pathways=diracpathways, phenotypes=as.factor(phenotypes)) })

   user  system elapsed
  0.892   0.000   0.893

> names(VarAnKendallV)[[1]]

[1] "DEATHPATHWAY"

> VarAnKendallV[[1]]

$E1
[1] 0.09441352

$E2
[1] 0.1310383

$E12
[1] 0.1187962

$zscore
[1] -3.711215

$zscoreD12D1
[1] 3.693266

$zscoreD12D2
[1] -4.005794

$VarEta1
[1] 3.480369e-05

$VarEta2
[1] 6.248694e-05

$sdtotal
[1] 0.009863601

$CovD12D12p
[1] 3.345665e-05

$CovD12D1p2
[1] 0.0006611348

$CovD1D12
```

```
[1] 0.0001258984

$CovD1D12
[1] 0.0001258984

$vartotD12D1
[1] 4.34854e-05

$vartotD12D2
[1] 9.239714e-06

$pvalue
[1] 0.0002062669

$pvalueD12D1
[1] 0.0002213919

$pvalueD12D2
[1] 6.180938e-05

$pvalueTotal
[1] 0.0001854281

$mysdtotal
[1] 0.01159303
```

The output consists of a list. Each element of the list corresponds to a pathway. The element
itself is a list. `E1` and `E2` are two fields which contain the measure of variability for phenotype
`levels(phenotypes)[1]` and `levels(phenotypes)[2]` respectively. Other list elements are
`pvalue` and `zscore` which are calculated through the theory of U-statistics and indicate the
statistical significance of the difference between $E1$ and $E2$.

3.3   Comparison of DIRAC and EVA

We ran the following code to compare statistics from DIRAC and from EVA.

```
> Nperm = 10;
> VarAnPerm = vector(mode="list",length=Nperm)
> for( i in seq_len(Nperm))
 {
    VarAnPerm[[i]] = GSReg.GeneSets.EVA(geneexpres=exprsdata, pathways=diracpathways,
                                        phenotypes=sample(phenotypes))
 }
> pvaluesperm = vector(mode="numeric",length=length(VarAnPerm[[1]]))
> for( i in seq_along(VarAnPerm[[1]]))
 {
    z = sapply(VarAnPerm,function(x) x[[i]]$E1 - x[[i]]$E2)
```

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```
    pvaluesperm[i] = mean(abs(VarAnKendallV[[i]]$E1-VarAnKendallV[[i]]$E2)<abs(z))
  }
>   zscore = sapply(VarAnKendallV,function(x) x$zscore);
>   pvalustat = sapply(VarAnKendallV,function(x) x$pvalue);
```

The figure represents that the theoretical p-value and p-value calculated from permutation test in EVA are very similar and we can use the theoretical p-value as a surrogate for p-value. Here is the histogram.

```
> hist(x=pvalustat,breaks=20,main="P-value Hist of U-Stat",xlim=c(0,1))
```

P−value Hist of U−Stat
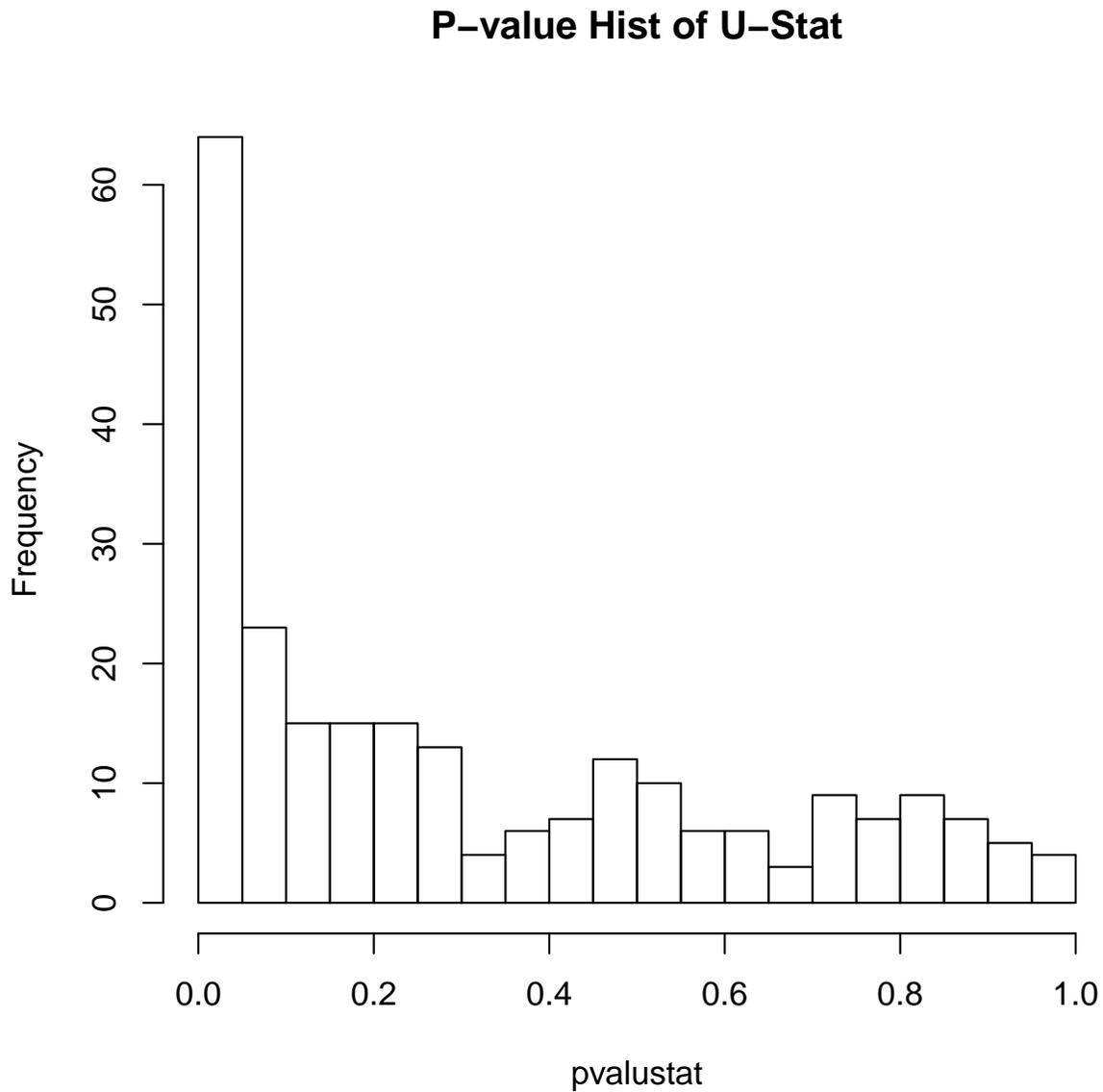
```
>  plot(x=abs(zscore),y=pvaluesperm,xlab="|Z-score|",
   ylab="p-value",col="red1",main="p-value comparisons")
>  zscorelin = seq(0,6,0.1);
>  pvaltheoretic = (1-pnorm(zscorelin))*2
>  lines(x=zscorelin,y=pvaltheoretic,type="l",pch=50,lty=5,col="darkblue")
>   legend("topright",legend=c("permutation test","U-Stat Estimation"),
          col=c("red","blue"),text.col=c("red","blue"),
   lty=c(NA,1),lwd=c(NA,2.5),pch=c(21,NA))
```
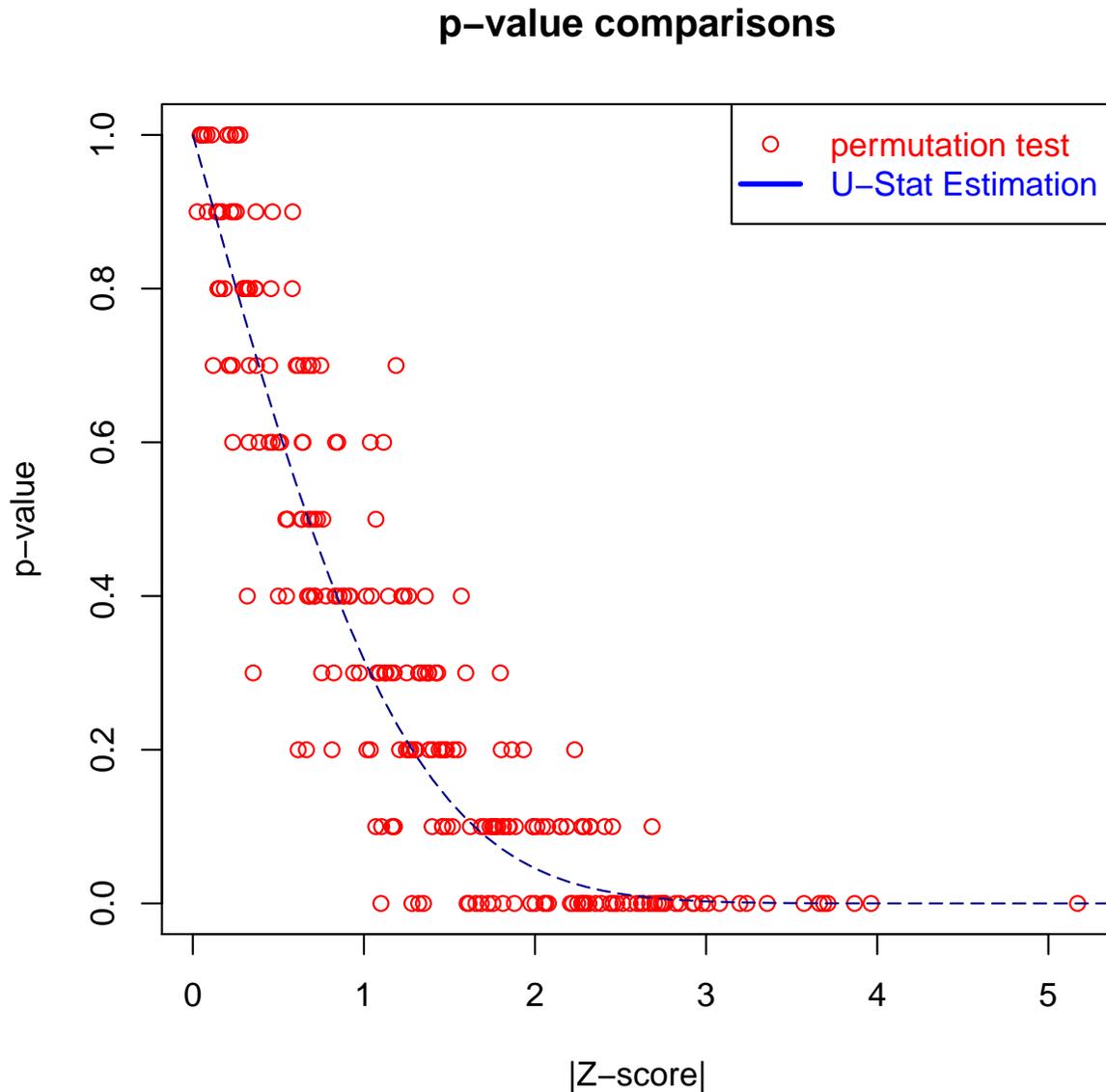

Figure 3: Comparing p-value from permutation test and U-statistic theory with only 10 permutations.
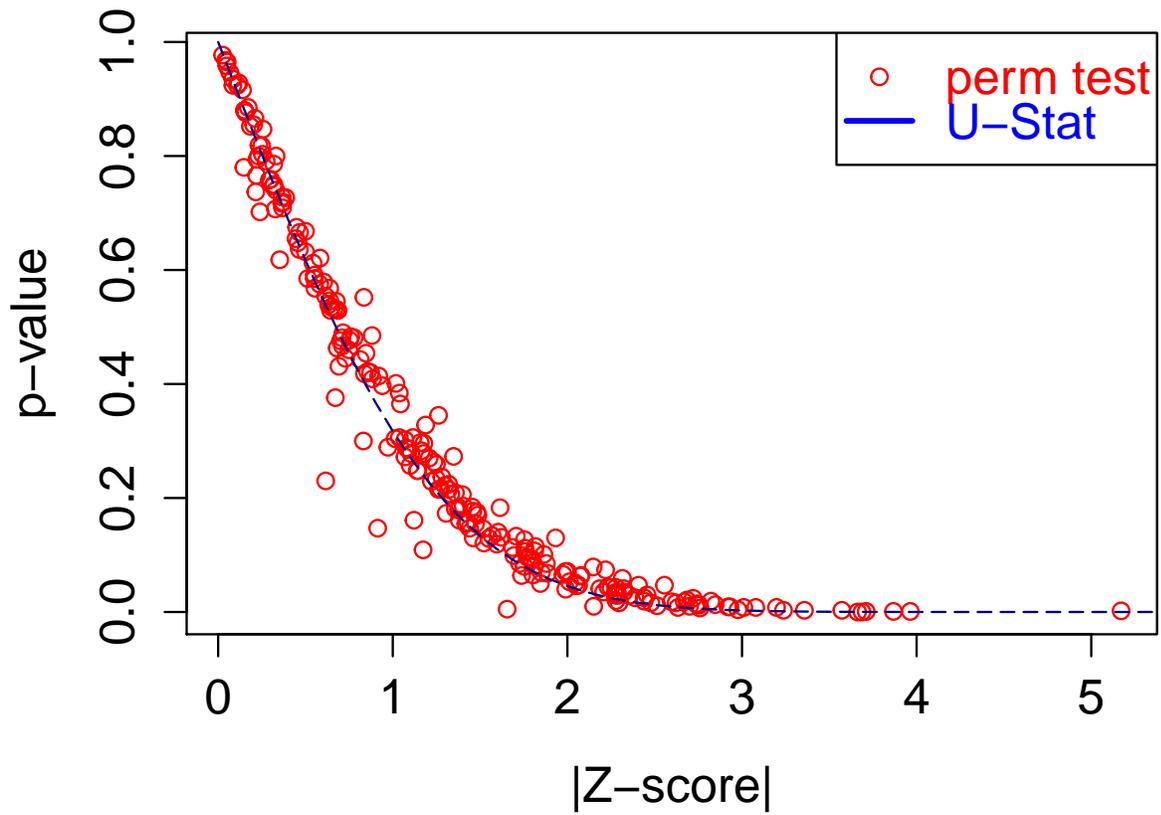
Figure 4: Theoretical p-value versus empirical p-value using 1000 permutations.

Figure 4 shows the result of comparing p-value EVA computing from 1000 permutation test and approximation using U-statistics theory (offline generated).

To compare with the p-value of the DIRAC analysis, we show the p-values of DIRAC versus U-Statistic methodology:

```
> plot(x=DIRACAn$pvalues,y=pvalustat,xlab ="DIRAC",
        ylab="EVA",main=sprintf("P-value Comparison corr=%2.2g",cor(x=DIRACAn$pvalues,y=pvalustat)))
> lmfit = lm(pvalustat~DIRACAn$pvalues-1)
> abline(lmfit)
> cor.test(x=DIRACAn$pvalues,y=pvalustat)

        Pearson's product-moment correlation

data:  DIRACAn$pvalues and pvalustat
t = 15.834, df = 238, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.6484375 0.7727928
sample estimates:
      cor
0.7162546
```

P−value Comparison corr=0.72
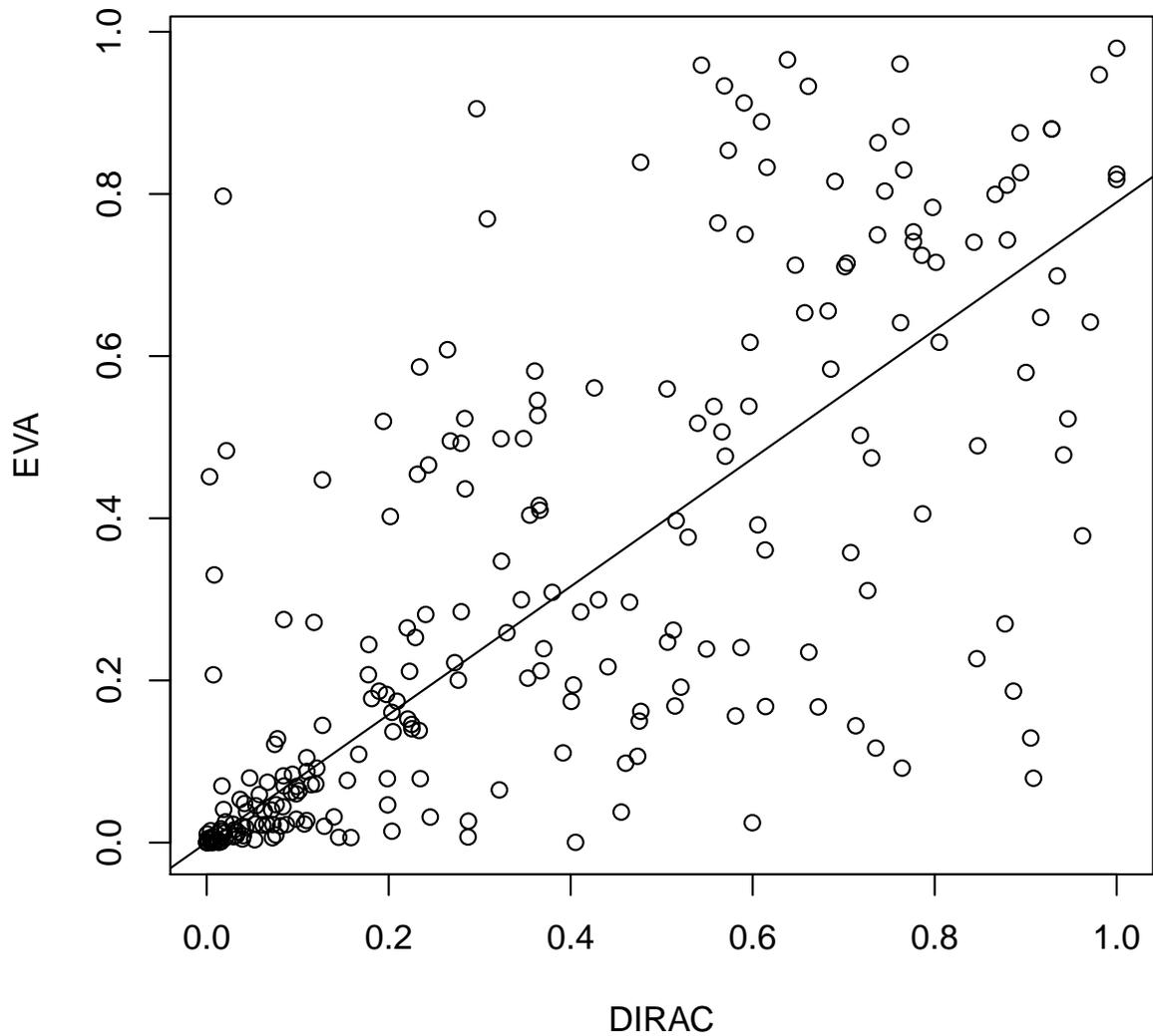
Also, the correlation of the p-values of DIRAC and U-Statistics is very high:

```
> cor(x=DIRACAn$pvalues,y=pvalustat)
[1] 0.7162546
```

If we use 1000 permutations instead of 10 permutations, we can see that the correlation is higher (0.88) as seen in Figure (5). The dysregulated pathways identified by $DIRAC$ are the following pathways:

```
 [1] "DEATHPATHWAY"        "NEUTROPHILPATHWAY"    "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"       "SKP2E2FPATHWAY"       "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"     "TGFBPATHWAY"          "PROTEASOMEPATHWAY"
[10] "MAPKPATHWAY"         "PDGFPATHWAY"          "BIOPEPTIDESPATHWAY"
[13] "SPPAPATHWAY"         "PYK2PATHWAY"          "MYOSINPATHWAY"
[16] "BETAOXIDATIONPATHWAY" "IL7PATHWAY"          "FMLPPATHWAY"
[19] "VITCBPATHWAY"        "CD40PATHWAY"          "CDC25PATHWAY"
[22] "MTORPATHWAY"         "RNAPATHWAY"           "FBW7PATHWAY"
[25] "LYMPHOCYTEPATHWAY"   "LAIRPATHWAY"          "HIVNEFPATHWAY"
[28] "ALKPATHWAY"          "P35ALZHEIMERSPATHWAY" "MSPPATHWAY"
[31] "GSK3PATHWAY"         "RELAPATHWAY"          "METPATHWAY"
[34] "TNFR2PATHWAY"        "AT1RPATHWAY"          "FREEPATHWAY"
[37] "ARAPPATHWAY"         "MRPPATHWAY"           "P53HYPOXIAPATHWAY"
[40] "IL18PATHWAY"         "STRESSPATHWAY"        "MEF2DPATHWAY"
[43] "STAT3PATHWAY"        "HSP27PATHWAY"         "EPONFKBPATHWAY"
[46] "NKCELLSPATHWAY"      "MONOCYTEPATHWAY"      "CARM_ERPATHWAY"
```

DIRAC and EVA have been shown mathematically similar. The main advantages of the EVA is efficiency in calculation as well as easier interpretation. Figure 5 a graphical example of such comparison. One can see that the p-values generated by DIRAC and EVA have high correlation, i.e. 0.88. Note that EVA is much faster than DIRAC. For example, in this case, we ran the computations on a Lenovo Thinkpad with Core(TM) i7-3720QM Intel CPU @2.6 GHz. For a thousand permutation, the DIRAC analysis took 207.47 seconds while the latter only took 0.3 seconds. Note that for multiple hypothesis adjustment, a thousand permutations may not be satisfactory and we require hundreds of thousand or a million permutation which may not be feasible.

Note that it is possible that some of the genes in a pathway are not represented in the expression data or are too short (e.g. less than 5 genes). Both `GSReg.GeneSets.EVA` and `GSReg.GeneSets.DIRAC` may ignore such pathways through parameter `minGeneNum`. Please see the manual for more details. If we the user wants to compare the results of DIRAC and

EVA, they can run the following code for plot DIRAC diagram of significantly perturbed pathways:

```
> DIRACAn =GSReg.GeneSets.DIRAC(exprsdata,diracpathways,phenotypes,Nperm=1000)


> significantPathwaysDIRAC = names(DIRACAn$mu1)[which(DIRACAn$pvalues<0.05)];
> mu1 = DIRACAn$mu1[significantPathwaysDIRAC];
> mu2 = DIRACAn$mu2[significantPathwaysDIRAC];
> #The dysregulated pathways
> names(mu1)

 [1] "DEATHPATHWAY"        "NEUTROPHILPATHWAY"    "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"       "SKP2E2FPATHWAY"       "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"     "TGFBPATHWAY"          "PROTEASOMEPATHWAY"
```
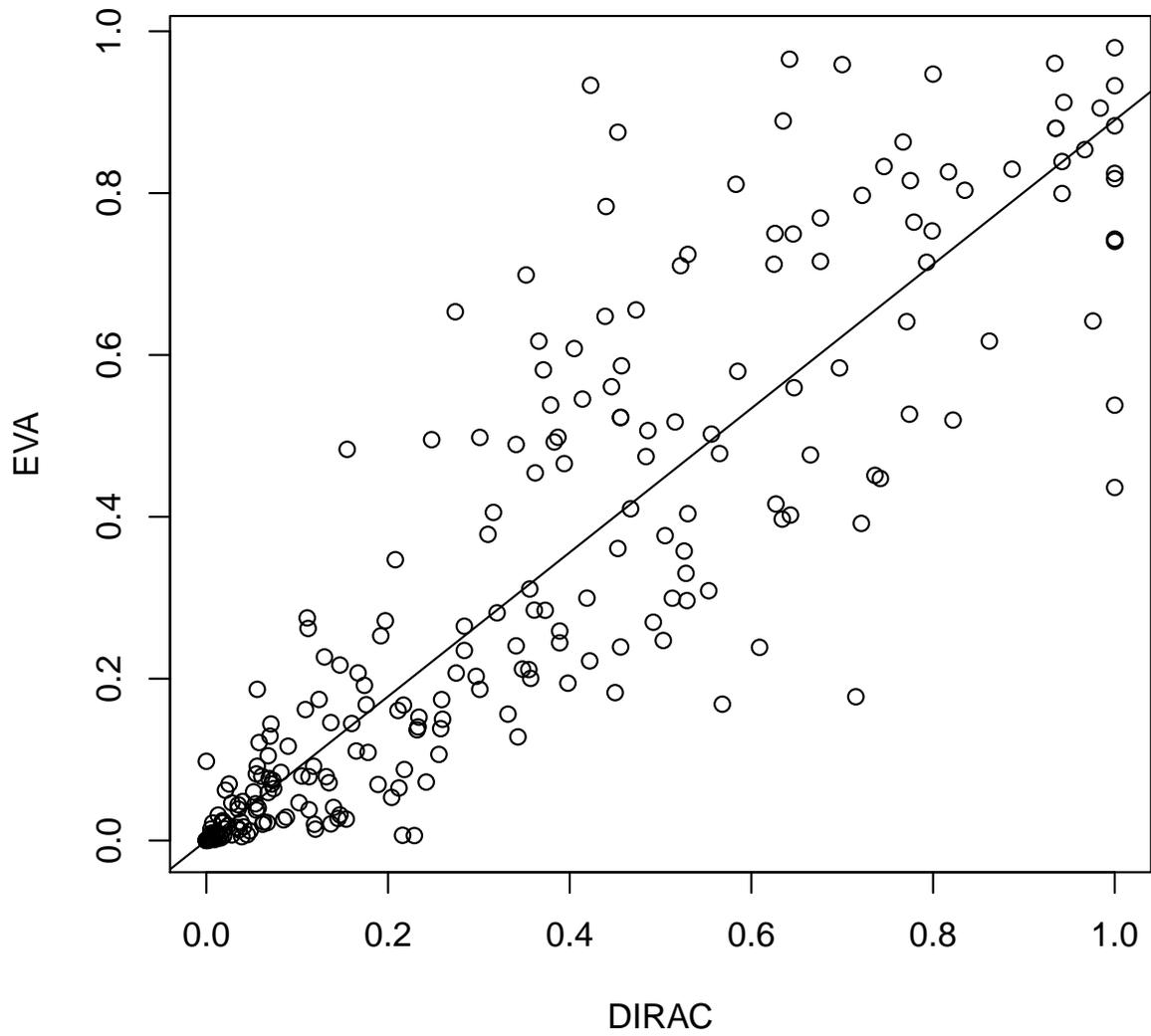
Figure 5: Comparing p-values EVA versus DIRAC. The correlation is 0.88.

```
 [10] "MAPKPATHWAY"          "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
 [13] "SPPAPATHWAY"           "PYK2PATHWAY"           "MYOSINPATHWAY"
 [16] "BETAOXIDATIONPATHWAY"  "IL7PATHWAY"            "FMLPPATHWAY"
 [19] "VITCBPATHWAY"          "CD40PATHWAY"           "CDC25PATHWAY"
 [22] "MTORPATHWAY"           "RNAPATHWAY"            "FBW7PATHWAY"
 [25] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"           "HIVNEFPATHWAY"
 [28] "ALKPATHWAY"            "P35ALZHEIMERSPATHWAY"  "MSPPATHWAY"
 [31] "GSK3PATHWAY"           "RELAPATHWAY"           "METPATHWAY"
 [34] "TNFR2PATHWAY"          "AT1RPATHWAY"           "FREEPATHWAY"
 [37] "ARAPPATHWAY"           "MRPPATHWAY"            "P53HYPOXIAPATHWAY"
 [40] "IL18PATHWAY"           "STRESSPATHWAY"         "MEF2DPATHWAY"
 [43] "STAT3PATHWAY"          "HSP27PATHWAY"          "EPONFKBPATHWAY"
 [46] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"

> plot(x=mu1,y=mu2,
     xlim=c(0,max(mu1,mu2)),ylim=c(0,max(mu1,mu2)),xlab="normal",ylab="disease",
     main="(a)  DIRAC significantly dysregulated pathways")
> lines(x=c(0,max(mu1,mu2)),y=c(0,max(mu1,mu2)))
```

(a) DIRAC significantly dysregulated pathways
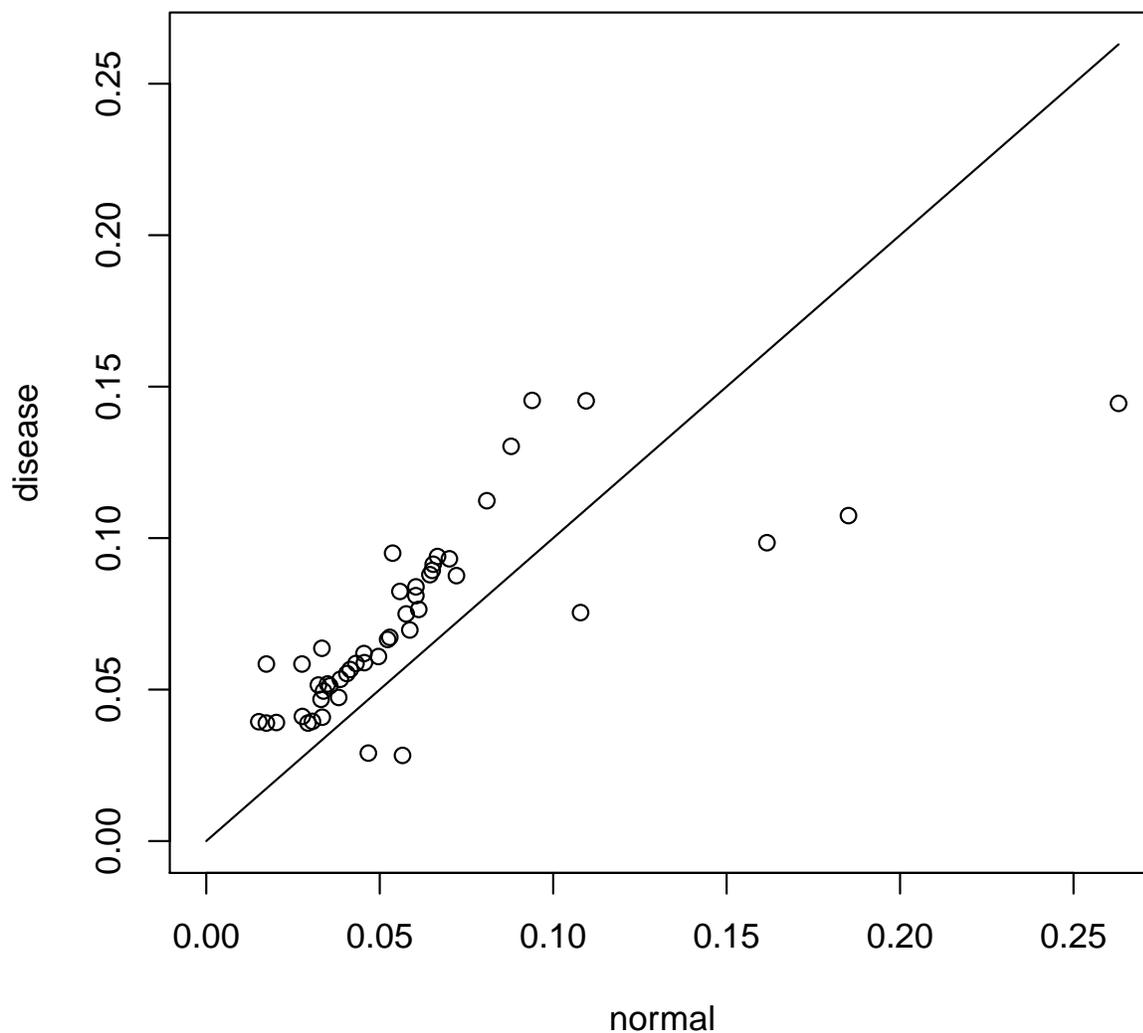
Now, if we do the analysis using EVA, we have:

```
> significantPathwaysGSV = names(which(pvalustat<0.05));

 [1] "DEATHPATHWAY"          "TCAPOPTOSISPATHWAY"    "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"          "TERCPATHWAY"           "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"   "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"     "TGFBPATHWAY"           "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"     "CDK5PATHWAY"           "MAPKPATHWAY"
[16] "NTHIPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"           "PYK2PATHWAY"           "CDC42RACPATHWAY"
```

```
[22] "MYOSINPATHWAY"          "BETAOXIDATIONPATHWAY" "IL7PATHWAY"
[25] "FMLPPATHWAY"            "FASPATHWAY"           "VITCBPATHWAY"
[28] "CD40PATHWAY"            "IGF1PATHWAY"          "CDC25PATHWAY"
[31] "MTORPATHWAY"            "RNAPATHWAY"           "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"      "LAIRPATHWAY"          "HIVNEFPATHWAY"
[37] "ALKPATHWAY"             "PEPIPATHWAY"          "MSPPATHWAY"
[40] "EDG1PATHWAY"            "GSK3PATHWAY"          "RELAPATHWAY"
[43] "METPATHWAY"             "TNFR2PATHWAY"         "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"         "GLYCOLYSISPATHWAY"    "TIDPATHWAY"
[49] "EPOPATHWAY"             "WNTPATHWAY"           "ARAPPATHWAY"
[52] "MRPPATHWAY"             "P53HYPOXIAPATHWAY"    "PITX2PATHWAY"
[55] "IL18PATHWAY"            "STRESSPATHWAY"        "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY"    "STAT3PATHWAY"         "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"         "MONOCYTEPATHWAY"      "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"

> eta1 = sapply(VarAnKendallV,function(x) x$E1)[significantPathwaysGSV];

        DEATHPATHWAY     TCAPOPTOSISPATHWAY    NEUTROPHILPATHWAY          PGC1APATHWAY
          0.09441352             0.08600289           0.35559678            0.04477053
         TERCPATHWAY            RARRXRPATHWAY        SKP2E2FPATHWAY  KERATINOCYTEPATHWAY
          0.07316017             0.06914038           0.03367003            0.07404055
     CHEMICALPATHWAY       METHIONINEPATHWAY           TGFBPATHWAY            PS1PATHWAY
          0.08521303             0.09913420           0.05028305            0.08080808
   PROTEASOMEPATHWAY             CDK5PATHWAY           MAPKPATHWAY           NTHIPATHWAY
          0.09617180             0.07975863           0.08504987            0.08091115
         PDGFPATHWAY      BIOPEPTIDESPATHWAY           SPPAPATHWAY           PYK2PATHWAY
          0.08698709             0.06767807           0.09690598            0.04711514
     CDC42RACPATHWAY           MYOSINPATHWAY BETAOXIDATIONPATHWAY            IL7PATHWAY
          0.08948195             0.09005280           0.02683983            0.10609668
         FMLPPATHWAY              FASPATHWAY          VITCBPATHWAY           CD40PATHWAY
          0.05736961             0.08676830           0.07888408            0.05294705
         IGF1PATHWAY            CDC25PATHWAY           MTORPATHWAY            RNAPATHWAY
          0.09115972             0.06265031           0.04188827            0.03009689
         FBW7PATHWAY       LYMPHOCYTEPATHWAY           LAIRPATHWAY        HIVNEFPATHWAY
          0.03174603             0.22390572           0.15222872            0.09020600
          ALKPATHWAY             PEPIPATHWAY           MSPPATHWAY           EDG1PATHWAY
          0.09130361             0.05194805           0.12150072            0.08801738
         GSK3PATHWAY             RELAPATHWAY           METPATHWAY          TNFR2PATHWAY
          0.11946928             0.06302309           0.10561315            0.05051566
         AT1RPATHWAY          ATRBRCAPATHWAY     GLYCOLYSISPATHWAY            TIDPATHWAY
          0.05943314             0.07166907           0.02308802            0.07331013
          EPOPATHWAY              WNTPATHWAY           ARAPPATHWAY            MRPPATHWAY
          0.07319696             0.10526414           0.05988456            0.04877345
    P53HYPOXIAPATHWAY            PITX2PATHWAY          IL18PATHWAY         STRESSPATHWAY
          0.07708666             0.09375387           0.13015873            0.05505364
        MEF2DPATHWAY     MITOCHONDRIAPATHWAY         STAT3PATHWAY        EPONFKBPATHWAY
          0.04399740             0.12572150           0.05179344            0.07910272
       NKCELLSPATHWAY         MONOCYTEPATHWAY        CARM_ERPATHWAY           HCMVPATHWAY
          0.07718643             0.25171192           0.06462137            0.07781385

> eta2 = sapply(VarAnKendallV,function(x) x$E2)[significantPathwaysGSV];
```

20

```
         DEATHPATHWAY     TCAPOPTOSISPATHWAY    NEUTROPHILPATHWAY           PGC1APATHWAY
           0.13103827             0.04531025           0.23546691             0.05588351
          TERCPATHWAY            RARRXRPATHWAY        SKP2E2FPATHWAY    KERATINOCYTEPATHWAY
           0.12770563             0.09090909           0.05856181             0.09013983
       CHEMICALPATHWAY       METHIONINEPATHWAY            TGFBPATHWAY             PS1PATHWAY
           0.11832612             0.15454545           0.07165057             0.12332852
      PROTEASOMEPATHWAY             CDK5PATHWAY            MAPKPATHWAY            NTHIPATHWAY
           0.13083213             0.10251869           0.10114801             0.09532055
           PDGFPATHWAY      BIOPEPTIDESPATHWAY            SPPAPATHWAY            PYK2PATHWAY
           0.11066711             0.08559859           0.12604711             0.06023958
       CDC42RACPATHWAY           MYOSINPATHWAY   BETAOXIDATIONPATHWAY             IL7PATHWAY
           0.11283954             0.11997526           0.06147186             0.13455988
           FMLPPATHWAY              FASPATHWAY           VITCBPATHWAY            CD40PATHWAY
           0.06840858             0.10725265           0.04252044             0.08041958
           IGF1PATHWAY            CDC25PATHWAY            MTORPATHWAY             RNAPATHWAY
           0.12087036             0.04413179           0.05994640             0.08719852
           FBW7PATHWAY        LYMPHOCYTEPATHWAY           LAIRPATHWAY          HIVNEFPATHWAY
           0.06307978             0.16065416           0.11574140             0.11884884
            ALKPATHWAY              PEPIPATHWAY            MSPPATHWAY            EDG1PATHWAY
           0.11181840             0.13593074           0.17344877             0.11010728
           GSK3PATHWAY             RELAPATHWAY            METPATHWAY           TNFR2PATHWAY
           0.16320909             0.08203463           0.12858234             0.07308378
          AT1RPATHWAY           ATRBRCAPATHWAY       GLYCOLYSISPATHWAY            TIDPATHWAY
           0.08115533             0.08771185           0.05112348             0.09458733
            EPOPATHWAY              WNTPATHWAY            ARAPPATHWAY             MRPPATHWAY
           0.09569080             0.12762130           0.07615440             0.08571429
      P53HYPOXIAPATHWAY           PITX2PATHWAY           IL18PATHWAY         STRESSPATHWAY
           0.09759247             0.11618223           0.18989899             0.07523998
          MEF2DPATHWAY     MITOCHONDRIAPATHWAY           STAT3PATHWAY         EPONFKBPATHWAY
           0.05508870             0.16778499           0.09647495             0.13372688
        NKCELLSPATHWAY          MONOCYTEPATHWAY        CARM_ERPATHWAY            HCMVPATHWAY
           0.09574739             0.17662338           0.08397641             0.08899711
> #The dysregulated pathways
> names(eta1)

 [1] "DEATHPATHWAY"          "TCAPOPTOSISPATHWAY"    "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"          "TERCPATHWAY"           "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"   "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"     "TGFBPATHWAY"           "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"     "CDK5PATHWAY"           "MAPKPATHWAY"
[16] "NTHIPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"           "PYK2PATHWAY"           "CDC42RACPATHWAY"
[22] "MYOSINPATHWAY"         "BETAOXIDATIONPATHWAY"  "IL7PATHWAY"
[25] "FMLPPATHWAY"           "FASPATHWAY"            "VITCBPATHWAY"
[28] "CD40PATHWAY"           "IGF1PATHWAY"           "CDC25PATHWAY"
[31] "MTORPATHWAY"           "RNAPATHWAY"            "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"           "HIVNEFPATHWAY"
[37] "ALKPATHWAY"            "PEPIPATHWAY"           "MSPPATHWAY"
[40] "EDG1PATHWAY"           "GSK3PATHWAY"           "RELAPATHWAY"
[43] "METPATHWAY"            "TNFR2PATHWAY"          "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"        "GLYCOLYSISPATHWAY"     "TIDPATHWAY"
```

```
[49] "EPOPATHWAY"          "WNTPATHWAY"         "ARAPPATHWAY"
[52] "MRPPATHWAY"          "P53HYPOXIAPATHWAY"  "PITX2PATHWAY"
[55] "IL18PATHWAY"         "STRESSPATHWAY"      "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY" "STAT3PATHWAY"       "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"      "MONOCYTEPATHWAY"    "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"

> plot(x=eta1,y=eta2,xlim=c(0,max(eta1,eta2)),ylim=c(0,max(eta1,eta2)),xlab="normal",ylab="disease",
      main="(b) EVA: Dysregulated pathways")

NULL

> lines(x=c(0,max(eta1,eta2)),y=c(0,max(eta1,eta2)))

NULL
```

(b) EVA: Dysregulated pathways
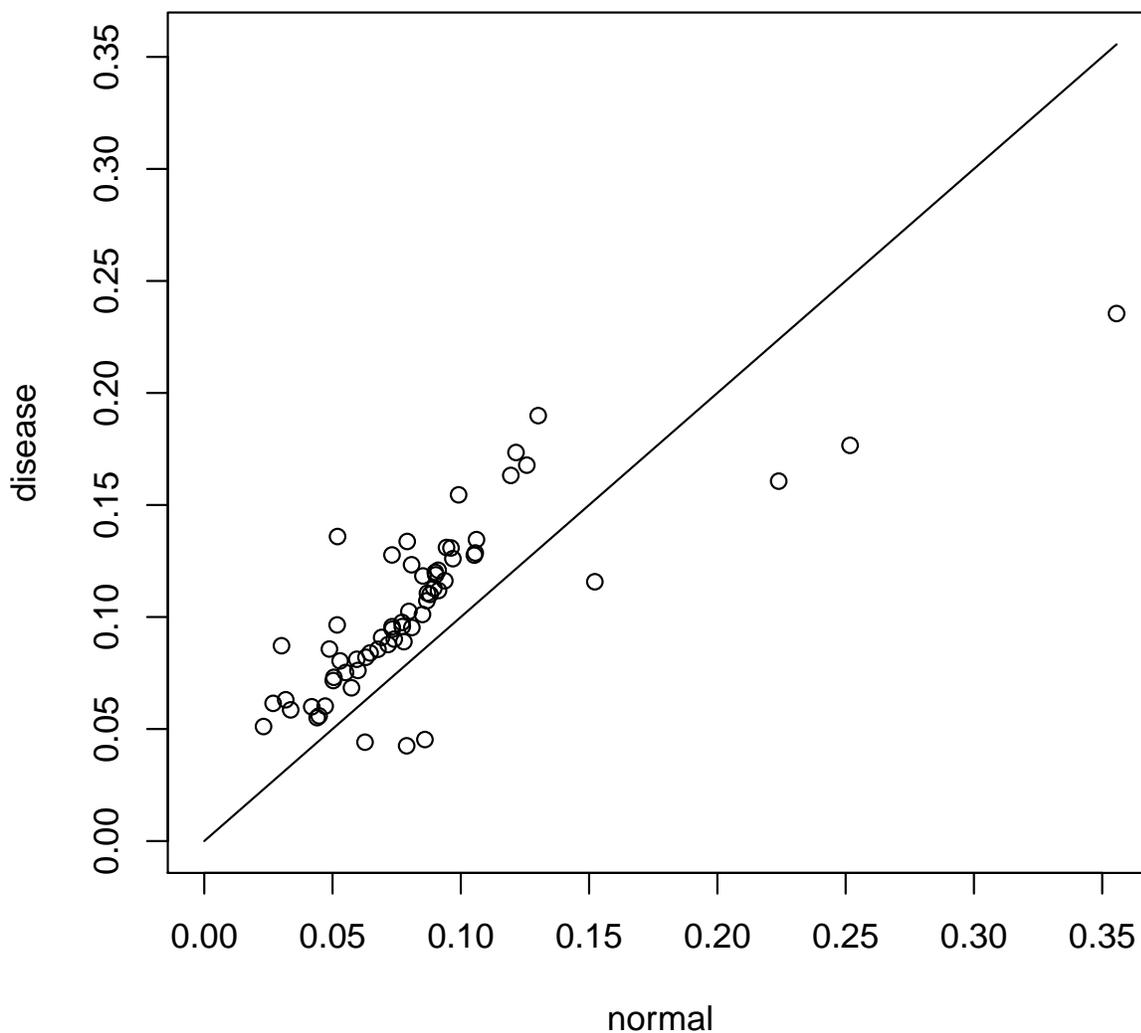
Although there is discrepancy in identified dysregulated pathways (p-value<0.05), the general trend found in [1] holds still true. The trend is that usually the dysregulated pathways have higher variability measure in more dangerous phenotypes. The figures reveal that both DIRAC and EVA have this property. DIRAC found 48 dysregulatd pathways and EVA discovered 64 pathways, 45 pathways showed up in both analysis, and 67 pathways were discovered totally.

```
> print(significantPathwaysGSV)
```

```
 [1] "DEATHPATHWAY"          "TCAPOPTOSISPATHWAY"     "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"          "TERCPATHWAY"            "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"    "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"     "TGFBPATHWAY"            "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"     "CDK5PATHWAY"            "MAPKPATHWAY"
[16] "NTHIPATHWAY"           "PDGFPATHWAY"            "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"           "PYK2PATHWAY"            "CDC42RACPATHWAY"
[22] "MYOSINPATHWAY"         "BETAOXIDATIONPATHWAY"  "IL7PATHWAY"
[25] "FMLPPATHWAY"           "FASPATHWAY"             "VITCBPATHWAY"
[28] "CD40PATHWAY"           "IGF1PATHWAY"            "CDC25PATHWAY"
[31] "MTORPATHWAY"           "RNAPATHWAY"             "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"            "HIVNEFPATHWAY"
[37] "ALKPATHWAY"            "PEPIPATHWAY"            "MSPPATHWAY"
[40] "EDG1PATHWAY"           "GSK3PATHWAY"            "RELAPATHWAY"
[43] "METPATHWAY"            "TNFR2PATHWAY"           "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"        "GLYCOLYSISPATHWAY"      "TIDPATHWAY"
[49] "EPOPATHWAY"            "WNTPATHWAY"             "ARAPPATHWAY"
[52] "MRPPATHWAY"            "P53HYPOXIAPATHWAY"      "PITX2PATHWAY"
[55] "IL18PATHWAY"           "STRESSPATHWAY"          "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY"   "STAT3PATHWAY"           "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"        "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"
 [1] "DEATHPATHWAY"          "TCAPOPTOSISPATHWAY"     "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"          "TERCPATHWAY"            "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"    "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"     "TGFBPATHWAY"            "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"     "CDK5PATHWAY"            "MAPKPATHWAY"
[16] "NTHIPATHWAY"           "PDGFPATHWAY"            "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"           "PYK2PATHWAY"            "CDC42RACPATHWAY"
[22] "MYOSINPATHWAY"         "BETAOXIDATIONPATHWAY"  "IL7PATHWAY"
[25] "FMLPPATHWAY"           "FASPATHWAY"             "VITCBPATHWAY"
[28] "CD40PATHWAY"           "IGF1PATHWAY"            "CDC25PATHWAY"
[31] "MTORPATHWAY"           "RNAPATHWAY"             "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"            "HIVNEFPATHWAY"
[37] "ALKPATHWAY"            "PEPIPATHWAY"            "MSPPATHWAY"
[40] "EDG1PATHWAY"           "GSK3PATHWAY"            "RELAPATHWAY"
[43] "METPATHWAY"            "TNFR2PATHWAY"           "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"        "GLYCOLYSISPATHWAY"      "TIDPATHWAY"
[49] "EPOPATHWAY"            "WNTPATHWAY"             "ARAPPATHWAY"
[52] "MRPPATHWAY"            "P53HYPOXIAPATHWAY"      "PITX2PATHWAY"
[55] "IL18PATHWAY"           "STRESSPATHWAY"          "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY"   "STAT3PATHWAY"           "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"        "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"
```

```
> print(significantPathwaysDIRAC)
```

```
 [1] "DEATHPATHWAY"          "NEUTROPHILPATHWAY"      "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"         "SKP2E2FPATHWAY"         "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"       "TGFBPATHWAY"            "PROTEASOMEPATHWAY"
[10] "MAPKPATHWAY"           "PDGFPATHWAY"            "BIOPEPTIDESPATHWAY"
[13] "SPPAPATHWAY"           "PYK2PATHWAY"            "MYOSINPATHWAY"
```

```
[16] "BETAOXIDATIONPATHWAY"   "IL7PATHWAY"            "FMLPPATHWAY"
[19] "VITCBPATHWAY"           "CD40PATHWAY"           "CDC25PATHWAY"
[22] "MTORPATHWAY"            "RNAPATHWAY"            "FBW7PATHWAY"
[25] "LYMPHOCYTEPATHWAY"      "LAIRPATHWAY"           "HIVNEFPATHWAY"
[28] "ALKPATHWAY"             "P35ALZHEIMERSPATHWAY" "MSPPATHWAY"
[31] "GSK3PATHWAY"            "RELAPATHWAY"           "METPATHWAY"
[34] "TNFR2PATHWAY"           "AT1RPATHWAY"           "FREEPATHWAY"
[37] "ARAPPATHWAY"            "MRPPATHWAY"            "P53HYPOXIAPATHWAY"
[40] "IL18PATHWAY"            "STRESSPATHWAY"         "MEF2DPATHWAY"
[43] "STAT3PATHWAY"           "HSP27PATHWAY"          "EPONFKBPATHWAY"
[46] "NKCELLSPATHWAY"         "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"
 [1] "DEATHPATHWAY"           "NEUTROPHILPATHWAY"     "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"          "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"        "TGFBPATHWAY"           "PROTEASOMEPATHWAY"
[10] "MAPKPATHWAY"            "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[13] "SPPAPATHWAY"            "PYK2PATHWAY"           "MYOSINPATHWAY"
[16] "BETAOXIDATIONPATHWAY"   "IL7PATHWAY"            "FMLPPATHWAY"
[19] "VITCBPATHWAY"           "CD40PATHWAY"           "CDC25PATHWAY"
[22] "MTORPATHWAY"            "RNAPATHWAY"            "FBW7PATHWAY"
[25] "LYMPHOCYTEPATHWAY"      "LAIRPATHWAY"           "HIVNEFPATHWAY"
[28] "ALKPATHWAY"             "P35ALZHEIMERSPATHWAY" "MSPPATHWAY"
[31] "GSK3PATHWAY"            "RELAPATHWAY"           "METPATHWAY"
[34] "TNFR2PATHWAY"           "AT1RPATHWAY"           "FREEPATHWAY"
[37] "ARAPPATHWAY"            "MRPPATHWAY"            "P53HYPOXIAPATHWAY"
[40] "IL18PATHWAY"            "STRESSPATHWAY"         "MEF2DPATHWAY"
[43] "STAT3PATHWAY"           "HSP27PATHWAY"          "EPONFKBPATHWAY"
[46] "NKCELLSPATHWAY"         "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"
```

3.4   Splice-EVA Analysis

SEVA needs junction overlap matrices. This can be done by function `GSReg.overlapJunction`.
Here is a piece of code which shows how to use this function.

```
> require('Homo.sapiens')

[1] TRUE

> require('org.Hs.eg.db')

[1] TRUE

> require('GenomicRanges')

[1] TRUE

> data(juncExprsSimulated)

[1] "juncExprsSimulated"

> overlapMat <- GSReg.overlapJunction(juncExprs =  junc.RPM.Simulated,
                                      geneexpr = geneExrsGSReg)
```

```
$Rest
$Rest$CMPK1
                           chr1:47799788-47834141 chr1:47834287-47838627
chr1:47799788-47834141                          1                      0
chr1:47834287-47838627                          0                      1
chr1:47838779-47840581                          0                      0
chr1:47838779-47840869                          0                      0
chr1:47840657-47840869                          0                      0
chr1:47840965-47842376                          0                      0
                           chr1:47838779-47840581 chr1:47838779-47840869
chr1:47799788-47834141                          0                      0
chr1:47834287-47838627                          0                      0
chr1:47838779-47840581                          1                      1
chr1:47838779-47840869                          1                      1
chr1:47840657-47840869                          0                      1
chr1:47840965-47842376                          0                      0
                           chr1:47840657-47840869 chr1:47840965-47842376
chr1:47799788-47834141                          0                      0
chr1:47834287-47838627                          0                      0
chr1:47838779-47840581                          0                      0
chr1:47838779-47840869                          1                      0
chr1:47840657-47840869                          1                      0
chr1:47840965-47842376                          0                      1


$Rest$CSF1
                             chr1:110453684-110456881 chr1:110457003-110458256
chr1:110453684-110456881                            1                        0
chr1:110457003-110458256                            0                        1
chr1:110458318-110459915                            0                        0
chr1:110460085-110464469                            0                        0
chr1:110464616-110465788                            0                        0
chr1:110464616-110466682                            0                        0
chr1:110466812-110467398                            0                        0
chr1:110467450-110467769                            0                        0
chr1:110467824-110468685                            0                        0
chr1:110467824-110471474                            0                        0
                             chr1:110458318-110459915 chr1:110460085-110464469
chr1:110453684-110456881                            0                        0
chr1:110457003-110458256                            0                        0
chr1:110458318-110459915                            1                        0
chr1:110460085-110464469                            0                        1
chr1:110464616-110465788                            0                        0
chr1:110464616-110466682                            0                        0
chr1:110466812-110467398                            0                        0
chr1:110467450-110467769                            0                        0
chr1:110467824-110468685                            0                        0
chr1:110467824-110471474                            0                        0
                             chr1:110464616-110465788 chr1:110464616-110466682
chr1:110453684-110456881                            0                        0
chr1:110457003-110458256                            0                        0
chr1:110458318-110459915                            0                        0
```

	chr1:110466812-110467398	chr1:110467450-110467769
chr1:110460085-110464469	0	0
chr1:110464616-110465788	1	1
chr1:110464616-110466682	1	1
chr1:110466812-110467398	0	0
chr1:110467450-110467769	0	0
chr1:110467824-110468685	0	0
chr1:110467824-110471474	0	0

	chr1:110466812-110467398	chr1:110467450-110467769
chr1:110453684-110456881	0	0
chr1:110457003-110458256	0	0
chr1:110458318-110459915	0	0
chr1:110460085-110464469	0	0
chr1:110464616-110465788	0	0
chr1:110464616-110466682	0	0
chr1:110466812-110467398	1	0
chr1:110467450-110467769	0	1
chr1:110467824-110468685	0	0
chr1:110467824-110471474	0	0

	chr1:110467824-110468685	chr1:110467824-110471474
chr1:110453684-110456881	0	0
chr1:110457003-110458256	0	0
chr1:110458318-110459915	0	0
chr1:110460085-110464469	0	0
chr1:110464616-110465788	0	0
chr1:110464616-110466682	0	0
chr1:110466812-110467398	0	0
chr1:110467450-110467769	0	0
chr1:110467824-110468685	1	1
chr1:110467824-110471474	1	1

$Rest$FAM72B

	chr1:120839865-120841975	chr1:120839985-120841975
chr1:120839865-120841975	1	1
chr1:120839985-120841975	1	1
chr1:120842052-120845995	0	0
chr1:120846119-120854492	0	0

	chr1:120842052-120845995	chr1:120846119-120854492
chr1:120839865-120841975	0	0
chr1:120839985-120841975	0	0
chr1:120842052-120845995	1	0
chr1:120846119-120854492	0	1

$Rest$KLHL12

	chr1:202861787-202862367	chr1:202861787-202863312
chr1:202861787-202862367	1	1
chr1:202861787-202863312	1	1
chr1:202862553-202863312	0	1
chr1:202863410-202863719	0	0
chr1:202863877-202864650	0	0
chr1:202863877-202878138	0	0
chr1:202864845-202865982	0	0

chr1:202866088-202878138	0	0
chr1:202878252-202880182	0	0
chr1:202880331-202887299	0	0
chr1:202887516-202888883	0	0
chr1:202889036-202894096	0	0
chr1:202894335-202896217	0	0
	chr1:202862553-202863312	chr1:202863410-202863719
chr1:202861787-202862367	0	0
chr1:202861787-202863312	1	0
chr1:202862553-202863312	1	0
chr1:202863410-202863719	0	1
chr1:202863877-202864650	0	0
chr1:202863877-202878138	0	0
chr1:202864845-202865982	0	0
chr1:202866088-202878138	0	0
chr1:202878252-202880182	0	0
chr1:202880331-202887299	0	0
chr1:202887516-202888883	0	0
chr1:202889036-202894096	0	0
chr1:202894335-202896217	0	0
	chr1:202863877-202864650	chr1:202863877-202878138
chr1:202861787-202862367	0	0
chr1:202861787-202863312	0	0
chr1:202862553-202863312	0	0
chr1:202863410-202863719	0	0
chr1:202863877-202864650	1	1
chr1:202863877-202878138	1	1
chr1:202864845-202865982	0	1
chr1:202866088-202878138	0	1
chr1:202878252-202880182	0	0
chr1:202880331-202887299	0	0
chr1:202887516-202888883	0	0
chr1:202889036-202894096	0	0
chr1:202894335-202896217	0	0
	chr1:202864845-202865982	chr1:202866088-202878138
chr1:202861787-202862367	0	0
chr1:202861787-202863312	0	0
chr1:202862553-202863312	0	0
chr1:202863410-202863719	0	0
chr1:202863877-202864650	0	0
chr1:202863877-202878138	1	1
chr1:202864845-202865982	1	0
chr1:202866088-202878138	0	1
chr1:202878252-202880182	0	0
chr1:202880331-202887299	0	0
chr1:202887516-202888883	0	0
chr1:202889036-202894096	0	0
chr1:202894335-202896217	0	0
	chr1:202878252-202880182	chr1:202880331-202887299
chr1:202861787-202862367	0	0
chr1:202861787-202863312	0	0

chr1:202862553-202863312	0	0
chr1:202863410-202863719	0	0
chr1:202863877-202864650	0	0
chr1:202863877-202878138	0	0
chr1:202864845-202865982	0	0
chr1:202866088-202878138	0	0
chr1:202878252-202880182	1	0
chr1:202880331-202887299	0	1
chr1:202887516-202888883	0	0
chr1:202889036-202894096	0	0
chr1:202894335-202896217	0	0

	chr1:202887516-202888883	chr1:202889036-202894096
chr1:202861787-202862367	0	0
chr1:202861787-202863312	0	0
chr1:202862553-202863312	0	0
chr1:202863410-202863719	0	0
chr1:202863877-202864650	0	0
chr1:202863877-202878138	0	0
chr1:202864845-202865982	0	0
chr1:202866088-202878138	0	0
chr1:202878252-202880182	0	0
chr1:202880331-202887299	0	0
chr1:202887516-202888883	1	0
chr1:202889036-202894096	0	1
chr1:202894335-202896217	0	0

	chr1:202894335-202896217
chr1:202861787-202862367	0
chr1:202861787-202863312	0
chr1:202862553-202863312	0
chr1:202863410-202863719	0
chr1:202863877-202864650	0
chr1:202863877-202878138	0
chr1:202864845-202865982	0
chr1:202866088-202878138	0
chr1:202878252-202880182	0
chr1:202880331-202887299	0
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chr1:33133999-33134340	0	0
chr1:33134455-33134573	0	0
chr1:33134733-33134832	0	0
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chr1:33135164-33138051	0	0
chr1:33138127-33138243	0	0
chr1:33138300-33138392	0	0
chr1:33138502-33145241	0	0
chr1:33116923-33117518	1	0

	chr1:33123173-33133826	chr1:33133999-33134340
chr1:33116923-33117515	0	0
chr1:33117662-33123028	0	0
chr1:33123173-33133826	1	0
chr1:33133999-33134340	0	1
chr1:33134455-33134573	0	0

chr1:33134733-33134832	0	0
chr1:33134958-33135087	0	0
chr1:33135164-33138051	0	0
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	chr1:33134455-33134573	chr1:33134733-33134832
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chr1:33116923-33117515	0	0
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	chr1:33138502-33145241	chr1:33116923-33117518
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chr1:33138127-33138243	0	0
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chr1:33116923-33117518	0	1

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chr1:205057943-205064115	1	1
chr1:205064192-205065810	0	0
chr1:205066039-205066454	0	0
chr1:205066523-205068117	0	0
chr1:205068234-205068869	0	0
chr1:205068940-205069039	0	0
chr1:205069192-205069280	0	0
chr1:205069399-205070728	0	0
chr1:205070837-205072985	0	0
chr1:205073147-205074156	0	0
chr1:205074296-205083917	0	0
chr1:205084033-205084986	0	0
chr1:205084089-205084986	0	0
chr1:205085011-205090983	0	0
chr1:205057940-205064001	1	1

	chr1:205064192-205065810	chr1:205066039-205066454
chr1:205057943-205064001	0	0
chr1:205057943-205064115	0	0
chr1:205064192-205065810	1	0
chr1:205066039-205066454	0	1
chr1:205066523-205068117	0	0
chr1:205068234-205068869	0	0
chr1:205068940-205069039	0	0
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chr1:205084089-205084986	0	0
chr1:205085011-205090983	0	0
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	chr1:205066523-205068117	chr1:205068234-205068869
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chr1:8426034-8482787	0	0	0
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chr1:226053667-226054274                              0                        0
chr1:226054382-226054812                              0                        0
chr1:226054863-226055588                              0                        0
chr1:226055730-226058776                              0                        0
chr1:226058813-226059687                              0                        0
chr1:226059753-226061988                              0                        0
chr1:226062067-226065095                              0                        0
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chr1:226067110-226070004                              0                        1


$Rest$TNFRSF1B
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chr1:12227226-12248853                         1                      0
chr1:12248952-12251014                         0                      1
chr1:12251142-12251831                         0                      0
chr1:12251980-12252488                         0                      0
chr1:12252581-12252920                         0                      0
chr1:12253155-12254012                         0                      0
chr1:12254089-12254641                         0                      0
chr1:12254675-12262024                         0                      0
chr1:12262228-12266797                         0                      0
chr1:12227226-12251014                         1                      1
                          chr1:12251142-12251831 chr1:12251980-12252488
chr1:12227226-12248853                         0                      0
chr1:12248952-12251014                         0                      0
chr1:12251142-12251831                         1                      0
chr1:12251980-12252488                         0                      1
chr1:12252581-12252920                         0                      0
chr1:12253155-12254012                         0                      0
chr1:12254089-12254641                         0                      0
chr1:12254675-12262024                         0                      0
chr1:12262228-12266797                         0                      0
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                          chr1:12252581-12252920 chr1:12253155-12254012
chr1:12227226-12248853                         0                      0
chr1:12248952-12251014                         0                      0
chr1:12251142-12251831                         0                      0
chr1:12251980-12252488                         0                      0
chr1:12252581-12252920                         1                      0
chr1:12253155-12254012                         0                      1
chr1:12254089-12254641                         0                      0
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chr1:12262228-12266797                         0                      0
chr1:12227226-12251014                         0                      0
                          chr1:12254089-12254641 chr1:12254675-12262024
chr1:12227226-12248853                         0                      0
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chr1:12251142-12251831                         0                      0
chr1:12251980-12252488                         0                      0
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chr1:12253155-12254012                           0                      0
chr1:12254089-12254641                           1                      0
chr1:12254675-12262024                           0                      1
chr1:12262228-12266797                           0                      0
chr1:12227226-12251014                           0                      0
                         chr1:12262228-12266797 chr1:12227226-12251014
chr1:12227226-12248853                           0                      1
chr1:12248952-12251014                           0                      1
chr1:12251142-12251831                           0                      0
chr1:12251980-12252488                           0                      0
chr1:12252581-12252920                           0                      0
chr1:12253155-12254012                           0                      0
chr1:12254089-12254641                           0                      0
chr1:12254675-12262024                           0                      0
chr1:12262228-12266797                           1                      0
chr1:12227226-12251014                           0                      1


$Rest$UQCRH
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chr1:46769492-46774773                           1                      0
chr1:46774799-46775827                           0                      1
chr1:46775716-46775827                           0                      1
chr1:46775988-46782224                           0                      0
chr1:46774799-46775569                           0                      1
                         chr1:46775716-46775827 chr1:46775988-46782224
chr1:46769492-46774773                           0                      0
chr1:46774799-46775827                           1                      0
chr1:46775716-46775827                           1                      0
chr1:46775988-46782224                           0                      1
chr1:46774799-46775569                           0                      0
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chr1:46769492-46774773                           0
chr1:46774799-46775827                           1
chr1:46775716-46775827                           0
chr1:46775988-46782224                           0
chr1:46774799-46775569                           1


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[1] "chr1:47799788-47834141" "chr1:47834287-47838627" "chr1:47838779-47840581"
[4] "chr1:47838779-47840869" "chr1:47840657-47840869" "chr1:47840965-47842376"

$genesJunction$CSF1
 [1] "chr1:110453684-110456881" "chr1:110457003-110458256" "chr1:110458318-110459915"
 [4] "chr1:110460085-110464469" "chr1:110464616-110465788" "chr1:110464616-110466682"
 [7] "chr1:110466812-110467398" "chr1:110467450-110467769" "chr1:110467824-110468685"
[10] "chr1:110467824-110471474"

$genesJunction$FAM72B
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 [1] "chr1:120839865-120841975" "chr1:120839985-120841975" "chr1:120842052-120845995"
 [4] "chr1:120846119-120854492"


$genesJunction$KLHL12
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  [4] "chr1:202863410-202863719" "chr1:202863877-202864650" "chr1:202863877-202878138"
  [7] "chr1:202864845-202865982" "chr1:202866088-202878138" "chr1:202878252-202880182"
 [10] "chr1:202880331-202887299" "chr1:202887516-202888883" "chr1:202889036-202894096"
 [13] "chr1:202894335-202896217"


$genesJunction$KLHL21
[1] "chr1:6653718-6655545" "chr1:6655617-6659107" "chr1:6659512-6659810"
[4] "chr1:6659512-6661857"


$genesJunction$MIR4632
[1] "chr1:12251142-12251831"


$genesJunction$MYSM1
  [1] "chr1:59125827-59126842" "chr1:59126899-59127078" "chr1:59127183-59131171"
  [4] "chr1:59131303-59132710" "chr1:59132898-59133519" "chr1:59133593-59134102"
  [7] "chr1:59133593-59134304" "chr1:59134238-59134656" "chr1:59134354-59134656"
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 [13] "chr1:59141252-59142598" "chr1:59142728-59147457" "chr1:59148217-59150825"
 [16] "chr1:59150923-59154710" "chr1:59154788-59155898" "chr1:59155921-59156012"
 [19] "chr1:59156089-59158533" "chr1:59158603-59160801" "chr1:59160879-59165657"


$genesJunction$NPHP4
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  [4] "chr1:5925333-5926433" "chr1:5926518-5927090" "chr1:5927175-5927800"
  [7] "chr1:5927956-5933312" "chr1:5933395-5934531" "chr1:5937358-5940174"
 [10] "chr1:5940299-5947346" "chr1:5947526-5950928" "chr1:5951088-5964677"
 [13] "chr1:5964864-5965352" "chr1:5965543-5965692" "chr1:5965843-5967175"
 [16] "chr1:5967282-5969212" "chr1:5969273-5987709" "chr1:5987847-5993207"
 [19] "chr1:5993389-6007164" "chr1:6007290-6008130" "chr1:6008311-6012760"
 [22] "chr1:6012896-6021854" "chr1:6022009-6027359" "chr1:6027423-6029147"
 [25] "chr1:6029319-6038330" "chr1:6038473-6046215" "chr1:6046387-6052304"
 [28] "chr1:5934717-5934934" "chr1:5965840-5967175" "chr1:5936583-5937153"
 [31] "chr1:5937358-5939406" "chr1:5939692-5940174"


$genesJunction$PEX14
[1] "chr1:10637247-10637871" "chr1:10659423-10678389" "chr1:10678474-10683076"
[4] "chr1:10683178-10684397" "chr1:10684494-10687329" "chr1:10687420-10689588"
[7] "chr1:10596354-10659295" "chr1:10596354-10678389"


$genesJunction$PIP5K1A
  [1] "chr1:151171557-151196721" "chr1:151171557-151196724" "chr1:151196755-151199796"
  [4] "chr1:151199876-151204147" "chr1:151204277-151204724" "chr1:151204841-151205027"
  [7] "chr1:151205179-151206673" "chr1:151206972-151209034" "chr1:151209239-151210658"
 [10] "chr1:151209239-151211606" "chr1:151210741-151211606" "chr1:151211654-151212431"
 [13] "chr1:151212515-151214599" "chr1:151212515-151219396" "chr1:151214745-151214914"
 [16] "chr1:151215043-151219396" "chr1:151219441-151220339" "chr1:151196755-151196847"
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[19] "chr1:151196882-151199796" "chr1:151212515-151214914"


$genesJunction$PSMB2
[1] "chr1:36040299-36042923" "chr1:36068975-36070834" "chr1:36070883-36074847"
[4] "chr1:36075009-36096875" "chr1:36096945-36101911" "chr1:36102033-36106943"
[7] "chr1:36043093-36070834"


$genesJunction$RBBP4
 [1] "chr1:33116923-33117515" "chr1:33117662-33123028" "chr1:33123173-33133826"
 [4] "chr1:33133999-33134340" "chr1:33134455-33134573" "chr1:33134733-33134832"
 [7] "chr1:33134958-33135087" "chr1:33135164-33138051" "chr1:33138127-33138243"
[10] "chr1:33138300-33138392" "chr1:33138502-33145241" "chr1:33116923-33117518"


$genesJunction$RBBP5
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 [4] "chr1:205066039-205066454" "chr1:205066523-205068117" "chr1:205068234-205068869"
 [7] "chr1:205068940-205069039" "chr1:205069192-205069280" "chr1:205069399-205070728"
[10] "chr1:205070837-205072985" "chr1:205073147-205074156" "chr1:205074296-205083917"
[13] "chr1:205084033-205084986" "chr1:205084089-205084986" "chr1:205085011-205090983"
[16] "chr1:205057940-205064001"


$genesJunction$RERE
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 [4] "chr1:8418976-8419824" "chr1:8420046-8420172" "chr1:8421550-8421823"
 [7] "chr1:8421936-8422743" "chr1:8422904-8424116" "chr1:8424315-8424806"
[10] "chr1:8424898-8425872" "chr1:8426034-8482787" "chr1:8482867-8525985"
[13] "chr1:8526083-8555123" "chr1:8555222-8557465" "chr1:8557589-8568686"
[16] "chr1:8568734-8601273" "chr1:8601377-8616534" "chr1:8616630-8617477"
[19] "chr1:8617582-8674620" "chr1:8674745-8684369" "chr1:8684439-8716032"
[22] "chr1:8716500-8813490" "chr1:8716500-8877219" "chr1:8482867-8483621"
[25] "chr1:8852647-8877219" "chr1:8716500-8852463"


$genesJunction$SLAMF7
[1] "chr1:160709146-160717984" "chr1:160709146-160719611" "chr1:160718304-160719611"
[4] "chr1:160719883-160720094" "chr1:160719922-160720094" "chr1:160720213-160721135"
[7] "chr1:160720213-160721976" "chr1:160721238-160721976" "chr1:160722038-160722896"


$genesJunction$SLC41A1
 [1] "chr1:205760846-205763998" "chr1:205764146-205764472" "chr1:205764606-205766052"
 [4] "chr1:205766131-205767032" "chr1:205767179-205767797" "chr1:205767943-205768085"
 [7] "chr1:205768229-205768887" "chr1:205768958-205770081" "chr1:205770188-205779198"
[10] "chr1:205780215-205781936"


$genesJunction$TFAP2E
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$genesJunction$TMEM51
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[4] "chr1:15480450-15536986" "chr1:15537058-15541391"


$genesJunction$TMEM63A
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  [4] "chr1:226036713-226037613" "chr1:226037780-226040365" "chr1:226040470-226041330"
  [7] "chr1:226041492-226043579" "chr1:226043641-226044353" "chr1:226044439-226044611"
 [10] "chr1:226044717-226046896" "chr1:226047049-226048560" "chr1:226048697-226049918"
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 [16] "chr1:226053667-226054274" "chr1:226054382-226054812" "chr1:226054863-226055588"
 [19] "chr1:226055730-226058776" "chr1:226058813-226059687" "chr1:226059753-226061988"
 [22] "chr1:226062067-226065095" "chr1:226065294-226066920" "chr1:226067110-226070004"


$genesJunction$TNFRSF1B
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  [7] "chr1:12254089-12254641" "chr1:12254675-12262024" "chr1:12262228-12266797"
 [10] "chr1:12227226-12251014"


$genesJunction$UQCRH
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[4] "chr1:46775988-46782224" "chr1:46774799-46775569"



$juncexpressed
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  [3] "chr1:47838779-47840581"   "chr1:47838779-47840869"
  [5] "chr1:47840657-47840869"   "chr1:47840965-47842376"
  [7] "chr1:110453684-110456881" "chr1:110457003-110458256"
  [9] "chr1:110458318-110459915" "chr1:110460085-110464469"
 [11] "chr1:110464616-110465788" "chr1:110464616-110466682"
 [13] "chr1:110466812-110467398" "chr1:110467450-110467769"
 [15] "chr1:110467824-110468685" "chr1:110467824-110471474"
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 [55] "chr1:59155921-59156012"   "chr1:59156089-59158533"
 [57] "chr1:59158603-59160801"   "chr1:59160879-59165657"
 [59] "chr1:5923465-5923950"     "chr1:5924093-5924398"
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[131] "chr1:33134733-33134832"  "chr1:33134958-33135087"
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[149] "chr1:205074296-205083917" "chr1:205084033-205084986"
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[159] "chr1:8421550-8421823"    "chr1:8421936-8422743"
[161] "chr1:8422904-8424116"    "chr1:8424315-8424806"
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[165] "chr1:8482867-8525985"     "chr1:8526083-8555123"
[167] "chr1:8555222-8557465"     "chr1:8557589-8568686"
[169] "chr1:8568734-8601273"     "chr1:8601377-8616534"
[171] "chr1:8616630-8617477"     "chr1:8617582-8674620"
[173] "chr1:8674745-8684369"     "chr1:8684439-8716032"
[175] "chr1:8716500-8813490"     "chr1:8716500-8877219"
[177] "chr1:8482867-8483621"     "chr1:8852647-8877219"
[179] "chr1:8716500-8852463"     "chr1:160709146-160717984"
[181] "chr1:160709146-160719611" "chr1:160718304-160719611"
[183] "chr1:160719883-160720094" "chr1:160719922-160720094"
[185] "chr1:160720213-160721135" "chr1:160720213-160721976"
[187] "chr1:160721238-160721976" "chr1:160722038-160722896"
[189] "chr1:205760846-205763998" "chr1:205764146-205764472"
[191] "chr1:205764606-205766052" "chr1:205766131-205767032"
[193] "chr1:205767179-205767797" "chr1:205767943-205768085"
[195] "chr1:205768229-205768887" "chr1:205768958-205770081"
[197] "chr1:205770188-205779198" "chr1:205780215-205781936"
[199] "chr1:15479280-15541391"   "chr1:15480450-15541391"
[201] "chr1:15541927-15545822"   "chr1:15480450-15536986"
[203] "chr1:15537058-15541391"   "chr1:226034633-226034735"
[205] "chr1:226034914-226036193" "chr1:226036255-226036598"
[207] "chr1:226036713-226037613" "chr1:226037780-226040365"
[209] "chr1:226040470-226041330" "chr1:226041492-226043579"
[211] "chr1:226043641-226044353" "chr1:226044439-226044611"
[213] "chr1:226044717-226046896" "chr1:226047049-226048560"
[215] "chr1:226048697-226049918" "chr1:226050051-226050155"
[217] "chr1:226050278-226050471" "chr1:226050551-226053597"
[219] "chr1:226053667-226054274" "chr1:226054382-226054812"
[221] "chr1:226054863-226055588" "chr1:226055730-226058776"
[223] "chr1:226058813-226059687" "chr1:226059753-226061988"
[225] "chr1:226062067-226065095" "chr1:226065294-226066920"
[227] "chr1:226067110-226070004" "chr1:12227226-12248853"
[229] "chr1:12248952-12251014"   "chr1:12251142-12251831"
[231] "chr1:12251980-12252488"   "chr1:12252581-12252920"
[233] "chr1:12253155-12254012"   "chr1:12254089-12254641"
[235] "chr1:12254675-12262024"   "chr1:12262228-12266797"
[237] "chr1:12227226-12251014"   "chr1:46769492-46774773"
[239] "chr1:46774799-46775827"   "chr1:46775716-46775827"
[241] "chr1:46775988-46782224"   "chr1:46774799-46775569"

> print(overlapMat[["Rest"]][["CMPK1"]])

                          chr1:47799788-47834141 chr1:47834287-47838627
chr1:47799788-47834141                         1                      0
chr1:47834287-47838627                         0                      1
chr1:47838779-47840581                         0                      0
chr1:47838779-47840869                         0                      0
chr1:47840657-47840869                         0                      0
chr1:47840965-47842376                         0                      0
                          chr1:47838779-47840581 chr1:47838779-47840869
chr1:47799788-47834141                         0                      0
```

```
chr1:47834287-47838627                            0                0
chr1:47838779-47840581                            1                1
chr1:47838779-47840869                            1                1
chr1:47840657-47840869                            0                1
chr1:47840965-47842376                            0                0
                        chr1:47840657-47840869 chr1:47840965-47842376
chr1:47799788-47834141                       0                      0
chr1:47834287-47838627                       0                      0
chr1:47838779-47840581                       0                      0
chr1:47838779-47840869                       1                      0
chr1:47840657-47840869                       1                      0
chr1:47840965-47842376                       0                      1
                        chr1:47799788-47834141 chr1:47834287-47838627
chr1:47799788-47834141                       1                      0
chr1:47834287-47838627                       0                      1
chr1:47838779-47840581                       0                      0
chr1:47838779-47840869                       0                      0
chr1:47840657-47840869                       0                      0
chr1:47840965-47842376                       0                      0
                        chr1:47838779-47840581 chr1:47838779-47840869
chr1:47799788-47834141                       0                      0
chr1:47834287-47838627                       0                      0
chr1:47838779-47840581                       1                      1
chr1:47838779-47840869                       1                      1
chr1:47840657-47840869                       0                      1
chr1:47840965-47842376                       0                      0
                        chr1:47840657-47840869 chr1:47840965-47842376
chr1:47799788-47834141                       0                      0
chr1:47834287-47838627                       0                      0
chr1:47838779-47840581                       0                      0
chr1:47838779-47840869                       1                      0
chr1:47840657-47840869                       1                      0
chr1:47840965-47842376                       0                      1

>
```

Another important function is kendall-τ restricted.

```
> V <- cbind(c(1,5,3),c(3,2,1))

     [,1] [,2]
[1,]    1    3
[2,]    5    2
[3,]    3    1

> rownames(V) <- c("F1","F2","F3")

[1] "F1" "F2" "F3"

> colnames(V) <- c("S1","S2")

[1] "S1" "S2"
```

```
> GSReg.kendall.tau.distance(V)

          S1        S2
S1 0.0000000 0.6666667
S2 0.6666667 0.0000000

> myRest1 <- cbind(c(0,1,1),c(1,0,1),c(1,1,0))

     [,1] [,2] [,3]
[1,]    0    1    1
[2,]    1    0    1
[3,]    1    1    0

> rownames(myRest1) <- rownames(V)

[1] "F1" "F2" "F3"

> colnames(myRest1) <- rownames(V)

[1] "F1" "F2" "F3"

> GSReg.kendall.tau.distance.restricted(V,myRest1)

          S1        S2
S1 0.0000000 0.6666666
S2 0.6666666 0.0000000

> GSReg.kendall.tau.distance(V)

          S1        S2
S1 0.0000000 0.6666667
S2 0.6666667 0.0000000

> myRest2 <- cbind(c(0,0,1),c(0,0,1),c(1,1,0))

     [,1] [,2] [,3]
[1,]    0    0    1
[2,]    0    0    1
[3,]    1    1    0

> rownames(myRest2) <- rownames(V)

[1] "F1" "F2" "F3"

> colnames(myRest2) <- rownames(V)

[1] "F1" "F2" "F3"

> GSReg.kendall.tau.distance.restricted(V,myRest2)

          S1        S2
S1 0.0000000 0.4999999
S2 0.4999999 0.0000000

> Temp1 <- cbind(c(0,1,1),c(0,0,0),c(0,1,0))
```

```
      [,1] [,2] [,3]
[1,]    0    0    0
[2,]    1    0    1
[3,]    1    0    0

> rownames(Temp1) <- rownames(V)

[1] "F1" "F2" "F3"

> colnames(Temp1) <- rownames(V)

[1] "F1" "F2" "F3"

> GSReg.kendall.tau.distance.template(V,Temp = Temp1)

        S1        S2
1.0000000 0.3333333
```

Now, we can use SEVA function and use the data from the paper [?].

```
> data(juncExprsSimulated)
> SEVAjunc <- GSReg.SEVA(juncExprs = junc.RPM.Simulated,
                         phenoVect = phenotypes,
                         geneexpr = geneExrsGSReg)
> print(sapply(SEVAjunc,function(x) x$pvalue))

      CMPK1        CSF1      FAM72B      KLHL12      KLHL21       MYSM1       NPHP4
1.00000000  1.00000000  0.00000000  0.00000000  0.19089848  0.00000000  0.53048443
      PEX14      PIP5K1A       PSMB2       RBBP4       RBBP5        RERE      SLAMF7
0.81734872  0.00000000  1.00000000  0.00000000  0.07123363  0.29801755  0.00000000
    SLC41A1       TMEM51      TMEM63A     TNFRSF1B       UQCRH
1.00000000  0.09424228  1.00000000  1.00000000  0.00000000

> #if you want to check Translational as well you can use 2 other p-values
> print(sapply(SEVAjunc,function(x) x$pvalueTotal))

      CMPK1        CSF1      FAM72B      KLHL12      KLHL21       MYSM1       NPHP4
1.00000000  1.00000000  0.00000000  0.00000000  0.26032517  0.00000000  1.00000000
      PEX14      PIP5K1A       PSMB2       RBBP4       RBBP5        RERE      SLAMF7
1.00000000  0.00000000  1.00000000  0.00000000  0.02343778  0.89405265  0.00000000
    SLC41A1       TMEM51      TMEM63A     TNFRSF1B       UQCRH
1.00000000  0.17473402  1.00000000  1.00000000  0.00000000

>
```

4   System Information

Session information:

```
> toLatex(sessionInfo())
```

- R version 3.4.2 (2017-09-28), `x86_64-pc-linux-gnu`

- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`

- Running under: `Ubuntu 16.04.3 LTS`

- Matrix products: default

- BLAS: `/home/biocbuild/bbs-3.6-bioc/R/lib/libRblas.so`

- LAPACK: `/home/biocbuild/bbs-3.6-bioc/R/lib/libRlapack.so`

- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils

- Other packages: AnnotationDbi 1.40.0, Biobase 2.38.0, BiocGenerics 0.24.0, GO.db 3.4.2, GSBenchMark 0.111.0, GSReg 1.12.0, GenomeInfoDb 1.14.0, GenomicFeatures 1.30.0, GenomicRanges 1.30.0, Homo.sapiens 1.3.1, IRanges 2.12.0, OrganismDbi 1.20.0, S4Vectors 0.16.0, TxDb.Hsapiens.UCSC.hg19.knownGene 3.2.2, org.Hs.eg.db 3.4.2

- Loaded via a namespace (and not attached): BiocInstaller 1.28.0, BiocParallel 1.12.0, Biostrings 2.46.0, DBI 0.7, DelayedArray 0.4.0, GenomeInfoDbData 0.99.1, GenomicAlignments 1.14.0, Matrix 1.2-11, R6 2.2.2, RBGL 1.54.0, RCurl 1.95-4.8, RMySQL 0.10.13, RSQLite 2.0, Rcpp 0.12.13, Rsamtools 1.30.0, SummarizedExperiment 1.8.0, XML 3.98-1.9, XVector 0.18.0, assertthat 0.2.0, biomaRt 2.34.0, bit 1.1-12, bit64 0.9-7, bitops 1.0-6, blob 1.1.0, compiler 3.4.2, digest 0.6.12, graph 1.56.0, grid 3.4.2, lattice 0.20-35, magrittr 1.5, matrixStats 0.52.2, memoise 1.1.0, pkgconfig 2.0.1, prettyunits 1.0.2, progress 1.1.2, rlang 0.1.2, rtracklayer 1.38.0, stringi 1.1.5, stringr 1.2.0, tibble 1.3.4, tools 3.4.2, zlibbioc 1.24.0

5   Literature Cited

References

[1] James A Eddy, Leroy Hood, Nathan D Price, and Donald Geman. Identifying tightly regulated and variably expressed networks by differential rank conservation (dirac). *PLoS computational biology*, 6(5):e1000792, 2010.

[2] Bahman Afsari. *Modeling cancer phenotypes with order statistics of transcript data*. PhD thesis, Johns Hopkins University, 2013.

[3] Maurice G Kendall. A new measure of rank correlation. *Biometrika*, 1938.