A probabilistic dynamical model for quantitative inference of the regulatory mechanism of transcription

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November 28, 2013

Abstract

Quantitative estimation of the regulatory relationship between transcription factors and genes is a fundamental stepping stone when trying to develop models of cellular processes. This task, however, is difficult for a number of reasons: transcription factors' expression levels are often low and noisy, and many transcription factors are post-transcriptionally regulated. It is therefore useful to infer the activity of the transcription factors from the expression levels of their target genes.

Contents

1 Installing the chipDyno package

The recommanded way to install the *Bioconductor* package *chipDyno* is to use the `biocLite` function available in the *Bioconductor* website. This way of installation should ensure that all the dependencies are met.

```
> source ("www.//bioconductor.org/chipDyno.R")
> biocLite("chipDyno")
```

2 Loading the package and getting help

The first step in any *chipDyno* analysis is to load the package. This package can be loaded when the *R* console is ready. At the *R* console type the following command:

```
> library("chipDyno")
```

Command `help` can be used to get help on any function. For example, to get help on the `chipDynoLikeStatGrad` type the following (both of them have the similar output):

```
> help(chipDynoLikeStatGrad)
> ?chipDynoLikeStatGrad
```

3 Input data and preparations

If the data is in Affymetrix CEL files then it may requered to do some preprocessing. This CEL files can be extracted using the bioconductor package *puma* [Pearson et al., 2009]. *puma* usually stores the point expressione in '*_exprs.csv' file and defferent levels of unicertainities in '*_se.csv' files as Comma-separated values.

Load the point expression levels data. Here the data was stored in comma-seperated value (csv) file.

```
> data_file =
+   "/home/muhammad/Dropbox/CElegans/cluster/3exp_15dp_After_meeting/eset_cElegans_exprs.csv"
> data_file

[1] "/home/muhammad/Dropbox/CElegans/cluster/3exp_15dp_After_meeting/eset_cElegans_exprs.csv"

> data_dictionary <- read.table(data_file, header = TRUE, sep = ",", quote = "",
+                     dec = ".", , na.strings = "NA", colClasses = NA, nrows = -1, skip = 0,
+                     check.names = TRUE, fill = TRUE, strip.white = FALSE,
+                     blank.lines.skip = TRUE, comment.char = "#",
+                     allowEscapes = FALSE, flush = FALSE)
> data_dictionary[is.na(data_dictionary)] <- 0
> probeId = data_dictionary[,1]
> as.matrix(probeId[1:7],,1)

     [,1]
[1,] "171720_x_at"
[2,] "171721_x_at"
[3,] "171722_x_at"
[4,] "171723_x_at"
[5,] "171724_x_at"
[6,] "171725_x_at"
[7,] "171726_x_at"
```

In our experimental data set there was 3 replication of the same experiment with 5 experimental time points with different environmental conditions. So there was (3x5=) 15 time points. We will to reorganize this datapoint maintaining the time series for different experiment. For our this example we will consider the data obtained from the second experiment.

```
> data = data_dictionary[,2:ncol(data_dictionary)]
> data_sample1 <- cbind(data[,10],data[,1],data[,4],data[,7],data[,13])
> data_sample2 <- cbind(data[,11],data[,2],data[,5],data[,8],data[,14])
> data_sample3 <- cbind(data[,12],data[,3],data[,6],data[,9],data[,15])
> data = data_sample2
> data_sample2[1:7, 1:5]
```

```
          [,1]      [,2]      [,3]      [,4]      [,5]
[1,]  8.485440  8.204751  7.733341  7.506436  7.654018
[2,] 10.458571  9.760224 10.237954 10.091678  9.138865
[3,]  9.866583 10.550956 10.569795 10.338855  9.695247
[4,] 14.022295 14.192518 14.114323 13.805791 13.372240
[5,] 10.827920 10.555282 10.269124 10.432771  9.820395
[6,] 13.728474 14.342469 14.197267 13.929742 12.964248
[7,]  5.981650  6.386036  5.598624  5.505857  5.542097
```

Load the the standard errors. The [Pearson et al., 2009] package creates many files with different percentile of posterior destribution we can choose anyone based on our requirement. Here as well we are considering the second experiment

```
> vars_file =
+   "/home/muhammad/Dropbox/CElegans/cluster/3exp_15dp_After_meeting/eset_cElegans_se.csv"
> vars_dictionary <- read.table(vars_file, header = TRUE, sep = ",", quote = "",
+                   dec = ".", , na.strings = "NA", colClasses = NA, nrows = -1, skip = 0,
+                   check.names = TRUE, fill = TRUE, strip.white = FALSE,
+                   blank.lines.skip = TRUE, comment.char = "#",
+                   allowEscapes = FALSE, flush = FALSE)
> vars_dictionary[is.na(vars_dictionary)] <- 0
> vars = vars_dictionary[,2:ncol(vars_dictionary)]
> vars_sample1 <- cbind(vars[,10],vars[,1],vars[,4],vars[,7],vars[,13])
> vars_sample2 <- cbind(vars[,11],vars[,2],vars[,5],vars[,8],vars[,14])
> vars_sample3 <- cbind(vars[,12],vars[,3],vars[,6],vars[,9],vars[,15])
> vars = vars_sample2 # Choose the sample
> vars_sample2[1:7, 1:5]
```

```
          [,1]      [,2]      [,3]      [,4]      [,5]
[1,] 0.1056184 0.1242523 0.1536280 0.1670771 0.1517485
[2,] 0.1499116 0.1990787 0.1652076 0.1722417 0.2492719
[3,] 0.1335496 0.1035425 0.1021652 0.1110768 0.1406269
[4,] 0.1139111 0.1038009 0.1044545 0.1171636 0.1376497
[5,] 0.1121100 0.1239257 0.1370347 0.1285520 0.1611539
[6,] 0.1476996 0.1264172 0.1261040 0.1390692 0.1899627
[7,] 0.4956338 0.4208474 0.6133670 0.6341569 0.5885441
```

Load the annotation file. From this annotation file we can find the gene names for corresponding probe Set ID. For annotation we used [Carlson,].

```
> annotation_file = "/home/muhammad/Dropbox/CElegans/annotation.txt"
> anno_dictionary <- read.table(annotation_file, header = FALSE, sep = "\t", quote = "",
+                   dec = ".", , na.strings = "NA", colClasses = NA, nrows = -1, skip = 0,
+                   check.names = TRUE, fill = TRUE, strip.white = FALSE,
+                   blank.lines.skip = TRUE, comment.char = "#",
+                   allowEscapes = FALSE, flush = FALSE)
> probeId2 = anno_dictionary[,1]
> geneName = anno_dictionary[,2]
> probeId=as.matrix(probeId,,1) # Probe set ID from experimental data
> probeId2=as.matrix(probeId2,,1) # Probe set ID from annotation file
> geneName=as.matrix(geneName,,1)
> geneName[1:7]
```

```
[1] "Y48E1B.14" "T01G9.2"   "F56B3.11"  "vit-4"     "cdc-25.1"  "col-160"   "cyp-23A1"
```

Remove the genes which expression level data at any time point is missing-

```
> index=array(0, dim<-c(nrow(data),1))
> for (i in 1: nrow(data)){
+   vec <- probeId[i]==probeId2
+         index[i]=colSums(vec)
+ }
> probeId=probeId[which(index!=0),];
> data=data[which(index!=0),];
> vars=vars[which(index!=0),];
```

For gene specific transcription factor activity we have consider the probablistic functional gene network Wormnet [Lee et al., 2008] and [Lee et al., 2010]. We have constructed a connectivity matrix where the columns represents the transcription factors and rows are the genes. For any element (i, j) of the connectivity matrix, if any transcription factor j can bind any specific gene i the value will be one, zero otherwise.

```
> connectivity_file =
+   "/home/muhammad/Dropbox/CElegans/Connectivity/connectivity_matHS_LC.txt"
> connectivity <- read.table(connectivity_file, header = FALSE, sep = ",", dec = ".",
+                 na.strings = "NA", colClasses = NA, nrows = -1, skip = 0,
+                 check.names = TRUE, fill = TRUE, strip.white = FALSE,
+                 blank.lines.skip = TRUE, comment.char = "#",
+                 allowEscapes = FALSE, flush = FALSE)
> probeId3 = as.matrix(connectivity[,1],,1) # Probe ID from connectivity files
> dataChip = connectivity[,2:ncol(connectivity)]
> dataChip[is.na(dataChip)] <- 0
```

Collect the common gene expression data-

```
> index=array(0, dim<-c(nrow(data),1))
> for (i in 1: nrow(data)){
+   vec <- geneName[i]==probeId3
+         index[i]=sum(vec)
+ }
> data=data[which(index!=0),];
> probeId=as.matrix(probeId[which(index!=0)]);
> geneName=as.matrix(geneName[which(index!=0)],,1);
> vars=vars[which(index!=0),];
```

Collect the common gene expression on dataChip-

```
> index=array(0, dim<-c(nrow(dataChip),1))
> for (i in 1: nrow(dataChip)){
+         vec <- probeId3[i]==geneName
+         index[i]=sum(vec)
+ }
> dataChip=dataChip[which(index!=0),];
> probeId3=as.matrix(probeId3[which(index!=0)],,1);
```

Avoid the duplicate entry

```
> tempIndex= duplicated(geneName)
> index=array(0, dim<-c(length(tempIndex),1))
> for (i in 1 : length(tempIndex)){
+         if (tempIndex[i]=="FALSE"){
+                 index[i]=1
+         }
+ }
```

```
> data=data[which(index!=0),];
> probeId=as.matrix(probeId[which(index!=0)]);
> geneName=as.matrix(geneName[which(index!=0)],,1);
> vars=vars[which(index!=0),];
> # Rearrange the dataChip data based on data entry
> dataChipNew = list()
> geneName2 = list()
> for (i in 1 : length(geneName)){
+         id = which (geneName[i]==probeId3)
+         dataChipNew = rbind(dataChipNew,dataChip[id,])
+         geneName2 = rbind(geneName2,probeId3[id,])
+ }
> #Create the binary matrix
> X= array(0, dim=c(nrow(dataChip),ncol(dataChip)))
> I <- c(which(dataChip>0))
> X[I] = 1

> file="/home/muhammad/Dropbox/CElegans/Connectivity/wTF2.1_test.txt"
> dictionaryTF <- read.table(file, header = TRUE, sep = "\t", quote = "", dec = ".",
+                 na.strings = "NA", colClasses = NA, nrows = -1, skip = 0,
+                 check.names = TRUE, fill = TRUE, strip.white = FALSE,
+                 blank.lines.skip = TRUE, comment.char = "#", allowEscapes = FALSE,
+                 flush = FALSE)
> TransNames = as.matrix(dictionaryTF[,1],,1)
```

Remove the transcription factors (if there any!) which has no evidence to transcribe genes and update the data.

```
> fakeX = rowSums(X)
> X=X[which(fakeX!=0),]
> annotation=geneName[which(fakeX!=0),] ### geneName has been renamed.
> annotation = matrix(annotation,,1)
> data= data[which(fakeX!=0),]
> vars= vars[which(fakeX!=0),]
```

Remove the genes which has no evidance to be transcribed by the given transcription factors and update the connectivity matrix.

```
> effectX=colSums(X)
> TransNames=TransNames[which(effectX!=0),]
> TransNames=matrix(TransNames,,1)
> X=X[,which(effectX!=0)]
```

Collect the number of active genes, transcription factors and assign a variable for the mean value of the transcription factor.

```
> nGenes= nrow(data)
> npts= ncol(data)
> nTrans = ncol(X)
> muIn = mat.or.vec(nTrans,1)
> muIn = matrix(muIn,,1)
```

4 Reduce the variables

The connectivity matrix is a huge sparse matrix. `chipReduceVariables` reduce number of variables in chipDyno model and return the same length integer vectors specifying the row, column indices of the non-zero entries and the corresponding value of the of the sparce matrix.

```
> source('/home/muhammad/Dropbox/Git/mlprojects/chipDyno/R/chipReduceVariables.R')
> R_C_V_nEffectGenes = chipReduceVariables(X);
> R = R_C_V_nEffectGenes[[1]]
> C = R_C_V_nEffectGenes[[2]]
> V = R_C_V_nEffectGenes[[3]]
> nEffectGenes = R_C_V_nEffectGenes[[4]]
> diagonal=matrix(mat.or.vec(1,nTrans),1,)
> diagonal[,]=0.5
> precs_mat=mat.or.vec(nrow(vars),ncol(vars))
> precs_mat[,]=1
> precs = precs_mat/(vars^2)
> beta=3;
> gamma=pi/4; # Initialize the degree of temporal continuity
> params=matrix(c(beta,gamma,t(muIn),0.1*t(V), diagonal),1,) # Rearran the parameters.
```

Setup the option variables for the Scaled Conjugate gradient optimization. `Options[1]` is set to 1 to display error values; If `Options[1]` is set to 0, then only warning messages are displayed. `Options[2]` is a measure of the absolute precision required for the value of X at the solution. The condition is satisfied when the absolute difference between the values of X between two successive steps is less than `Options[2]`. `Options[3]` is a measure of the precision required of the objective function at the solution. This condition is satisfied when the absolute difference between the objective function values between two successive steps is less than `Options[3]`. Both this and the condition for `Options[2]` must be satisfied for termination. `Options[14]` is the maximum number of iterations; default value is 100.

```
> options = array(0, dim=c(1,18))
> options[1]=1;
> options[2]=0.0001
> options[3]=0.0001
> options[14]=1500 # No of iteration
> options[17]=0.1

> #% Temporary load functions
> source("/home/muhammad/Dropbox/Git/mlprojects/chipDyno/R/chipDynoLikeStatNoise.R")
> source("/home/muhammad/Dropbox/Git/mlprojects/chipDyno/R/chipDynoLikeStatNoiseGrad.R")
> source("/home/muhammad/Dropbox/Git/mlprojects/chipDyno/R/SCGoptimNoise.R")
```

5 Optimization of Likelihood

`chipDynoLikeStat` and `chipDynoLikeStatNoise` find out the marginal likelihood for chipDyno dynamical model without and with the uncertainty of the expression level respectively. The format of the function is-

```
> options[14]=1000
> # CHIPDYNOLIKESTATNOISE marginal likelihood for chipChip dynamical model
> # FORMAT chipDynoLikeStatNoise <- function(params,data,precs,X,nEffectGenes,R,C)
> # DESC compute the marginal likelihood for chipChip dynamical model
> # ARG params: concatenated vector of multiple parameters(beta, gamma,
> # initial mean of the transcription factors, and
> # a vector to create diagonal matrix used to reduce the sparsity of covariance)
> # ARG data : point estimate of the expression level
> # ARG precs : uncertainty of the expression level
> # ARG X : connectivity measurement between genes and transcription factors
> # ARG nEffectGenes : effectice gene name
> # ARG R, C : same length integer vectors specifying the row and column
> # indices of the non-zero entries of the sparce matrix
> # RETURN likelihood : marginal likelihood
```

6 Gradiant Optimization

chipDynoLikeStatGrad and chipDynoLikeStatNoiseGrad calculate the gradient of chipDyno dynamical model without and with the uncertainty of the expression level respectively. The format of the function is-

```
> options[14]=5
> # FORMAT chipDynoLikeStatNoiseGrad <- function(params, data, precs, X, nEffectGenes, R, C)
> # DESC compute the gradient of chipDynoLikeStatNoise for chipChip dynamical model
> # ARG params: concatenated vector of multiple parameters(beta, gamma,
> # initial mean of the transcription factors, and
> # a vector to create diagonal matrix used to reduce the sparsity of covariance)
> # ARG data : point estimate of the expression level
> # ARG precs : uncertainty of the expression level
> # ARG X : connectivity measurement between genes and transcription factors
> # ARG nEffectGenes : effectice gene name
> # ARG R, C : same length integer vectors specifying the row and column
> # indices of the non-zero entries of the sparce matrix
> # RETURN f : list of beta,gamma, mu, Sigma, diagonal
```

7 Parameter Optimization

For marameter optimization we have used Scaled conjugate gradient optimization technique. This was done by Nabney et. al. for the matlab tool box Netlab. Here we have created the R version of SCG optimization.

```
Cycle : 1         Error=  4769.431         Scale=  1
Cycle : 2         Error=  3435.412         Scale=  0.5
Cycle : 3         Error=  3040.608         Scale=  0.25
Cycle : 4         Error=  2395.916         Scale=  0.125
Cycle : 5         Error=  2004.683         Scale=  0.0625
[1] "Warning: Maximum number of iterations has been exceeded"
```

Collect the optimized values of mean, degree of temporal continuity, gradient and construct the optimized sparse matrix.

```
> library(Matrix)
> V=params[(3+nTrans):(length(params)-nTrans)]
> preSigma <- sparseMatrix(R, C, x=V, dims = c(nEffectGenes,nTrans))
> diagonal = params[(length(params)-nTrans+1) :(length(params))];
> Sigma = t(preSigma)%*%preSigma + diag(diagonal*diagonal)
> beta = params[1]
> gamma = params[2]
> mu = params[3:(2+nTrans)]
```

Save the data for the further analysis.

```
> save.image("OptimizedResults_chipDyno2.RData")
```

```
> library(Matrix)
> V=params[(3+nTrans):(length(params)-nTrans)]
> preSigma <- sparseMatrix(R, C, x=V, dims = c(nEffectGenes,nTrans))
> diagonal = params[(length(params)-nTrans+1) :(length(params))];
> Sigma = t(preSigma)%*%preSigma + diag(diagonal*diagonal)
> Sigma[1,7:1,7]
```

```
[1] 0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.0000000 0.2855354
```

```
> beta = params[1]
> beta

[1] 2.956656

> gamma = params[2]
> gamma

[1] 1.002117

> mu = params[3:(2+nTrans)]
> mu

 [1]  0.0061301293  0.0140728862  0.0122150177  0.0135938941  0.0004971842  0.0120905132
 [7]  0.0440501921  0.0376756092  0.0315684362  0.0025020464  0.0004971842  0.0127967696
[13]  0.0298659677  0.0036280829  0.0137589917  0.0324063555  0.0111784321  0.0133022331
[19]  0.0004971842  0.0170766473  0.0036280829  0.0086353393  0.0071787549 -0.0133753124
[25]  0.0014286937  0.0714498366  0.0001107559  0.0143491183  0.0176310382  0.0001107559
[31]  0.0034806406  0.0036280829  0.1326625095  0.0065212583  0.0018202303
```

8 Plot mean

The following figure represent the mean value of genes.

9 Session info

Here is the output of `sessionInfo` on the system on which this document was compiled:

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

- R version 3.0.2 (2013-09-25), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=en_GB.UTF-8, LC_NUMERIC=C, LC_TIME=en_GB.UTF-8, LC_COLLATE=en_GB.UTF-8, LC_MONETARY=en_GB.UTF-8, LC_MESSAGES=en_GB.UTF-8, LC_PAPER=en_GB.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_GB.UTF-8, LC_IDENTIFICATION=C
- Base packages: base, datasets, graphics, grDevices, methods, stats, utils
- Other packages: Matrix 1.1-0
- Loaded via a namespace (and not attached): BiocStyle 0.0.19, grid 3.0.2, lattice 0.20-24, tools 3.0.2

References

[Carlson,] Carlson, M. *celegans.db: Affymetrix celegans annotation data (chip celegans)*. R package version 2.9.0.

[Lee et al., 2008] Lee, I., Lehner, B., Crombie, C., Wang, W., Fraser, A. G., and Marcotte, E. M. (2008). A single network comprising the majority of genes accurately predicts the phenotypic effects of gene perturbation in c. elegans. *Nature Genetics*, 40:181–188.

[Lee et al., 2010] Lee, I., Lehner, B., Vavouri, T., Shin, J., Fraser, A. G., and Marcotte, E. M. (2010). Predicting genetic modifier loci using functional gene networks. *Genome Research*, pages 1143–1153. 20(8).

[Pearson et al., 2009] Pearson, R., Liu, X., Sanguinetti, G., Milo, M., Lawrence, N., and Rattray, M. (2009). puma: a bioconductor package for propagating uncertainty in microarray analysis. *BMC Bioinformatics*. 10:211.

```
> par(mfrow=c(2,1))
> plot(mu, col="green", type="l")
> #barplot(height=sample(1:10,5), names=LETTERS[1:5], col=1:5)
> barplot(mu,  col=c("blue"))
```

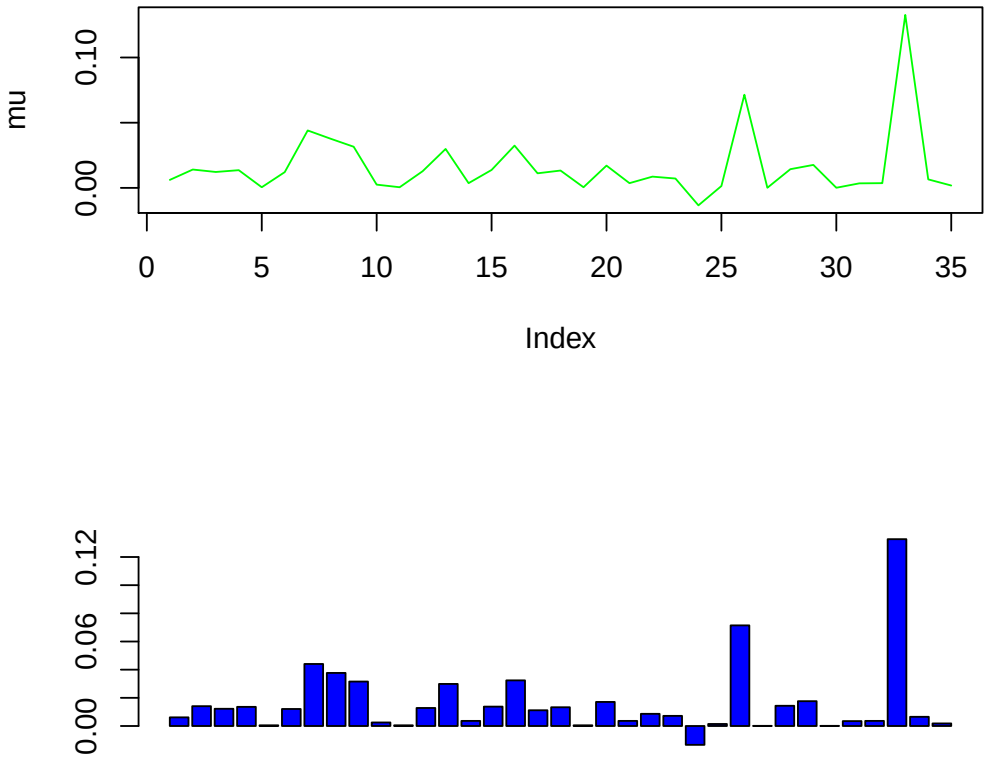

Figure 1: Plot mean value