xseq – assessing functional impact on gene expression of mutations in cancer

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Introduction

The xseq model specifies how the expression Y of a group of genes in a patient is influenced by the somatic mutation status of a gene g in the patient. The main question we address is whether gene g co-associates with disrupted expression to itself or its connected genes as defined by an influence graph. This concept is motivated by biological hypotheses predicting that some functional mutations will exhibit a "transcriptional shadow", resulting from a mechanistic impact on the gene expression profile of a tumour. For example, loss-of-function mutations (nonsense mutations, frame-shifting indels, splice-site mutations or homozygous copy number deletions) occurring in tumour suppressor genes like *TP53* can cause loss of expression due to nonsense-mediated mRNA decay or gene dosage effects. In this context, we define a *cis-effect* as a genetic or epigenetic aberration that results in up-regulation or down-regulation of the gene itself. In contrast, some mutations can disrupt the expression of other genes in the same biochemical pathway (*trans-effects*). This class of mutations tends to cast a long transcriptional shadow over many genes across the genome. β-catenin (*CTNNB1*) mutations, which drive constitutive activation of Wnt signalling in several cancer types, are a potent example of mutational impact on gene expression.

Inputs

The `xseq` model is predicated on the idea that mutations with functional effects on transcription will exhibit measurable signals in mRNA transcripts biochemically related to the mutated gene –thus imposing a transcriptional shadow across part (or all) of a pathway. To infer this property, three key inputs are required for the model: a patient-gene matrix encoding the presence/absence of a mutation (any form of somatic genomic aberrations that can be ascribed to a gene, e.g., SNVs, indels, or copy number alterations); a patient-gene expression matrix encoding continuous value expression data (e.g., from RNASeq or microarrays); and a graph structure encoding whether two genes are known to be functionally related (e.g., obtained through literature, databases, or co-expression data). `xseq` uses a precomputed 'influence graph' as a means to incorporate prior gene-gene relationship knowledge into its modelling framework. For analysis of mutation impact in-*cis*, the graph reduces to the simple case where the mutated gene is only connected to itself.

```
library(xseq)
data(mut, expr, cna.call, cna.logr, net)

mut[1:5,1:5]
```

```
##            sample hgnc_symbol entrezgene variant_type chrom
## 1 TCGA-AB-2802-03       TBX15          0     MISSENSE     1
## 2 TCGA-AB-2802-03      TCHHL1          0     MISSENSE     1
## 3 TCGA-AB-2802-03    ANKRD30A          0     MISSENSE    10
## 4 TCGA-AB-2802-03      PTPN11          0     MISSENSE    12
## 5 TCGA-AB-2802-03       EP400          0   SYNONYMOUS    12
```

```
expr[1:5,1:5]
```

```
##                      NPM1    RUNX1     KDM6A     FLT3     TP53
## TCGA-AB-3007-03 13.19168 13.99527 10.178914 12.50839 11.13443
## TCGA-AB-2990-03 13.64727 14.02808 10.099283 13.46506 10.98983
## TCGA-AB-2915-03 12.60635 13.45520 11.060389 12.23997 10.98188
## TCGA-AB-2927-03 12.61067 14.48913 10.743187 13.54870 10.89469
## TCGA-AB-3000-03 13.26361 11.88463  9.955398 13.40446 11.56106
```

```
cna.call[1:5,1:5]
```

```
##                 NPM1 RUNX1 KDM6A FLT3 TP53
## TCGA-AB-2803-03    0     0     0    0    0
## TCGA-AB-2804-03    0     0     0    0    0
## TCGA-AB-2805-03    0     0     0    0    0
## TCGA-AB-2806-03    0     0     0    0    0
## TCGA-AB-2807-03    0     1     0    0    0
```

```
cna.logr[1:5,1:5]
```

```
##                    NPM1   RUNX1   KDM6A    FLT3    TP53
## TCGA-AB-2884-03 -0.0065  0.0056  0.0110 -0.0023 -0.0015
## TCGA-AB-2943-03 -0.0878 -0.0800  0.0026 -0.0527 -0.1136
## TCGA-AB-2938-03  0.0384  0.0004 -0.0044  0.0183  0.9600
## TCGA-AB-2806-03  0.0017 -0.0062 -0.0005  0.0011  0.0194
## TCGA-AB-2826-03  0.0230  0.0123  0.0069  0.0095  0.0091
```

```
net[1:2]
```

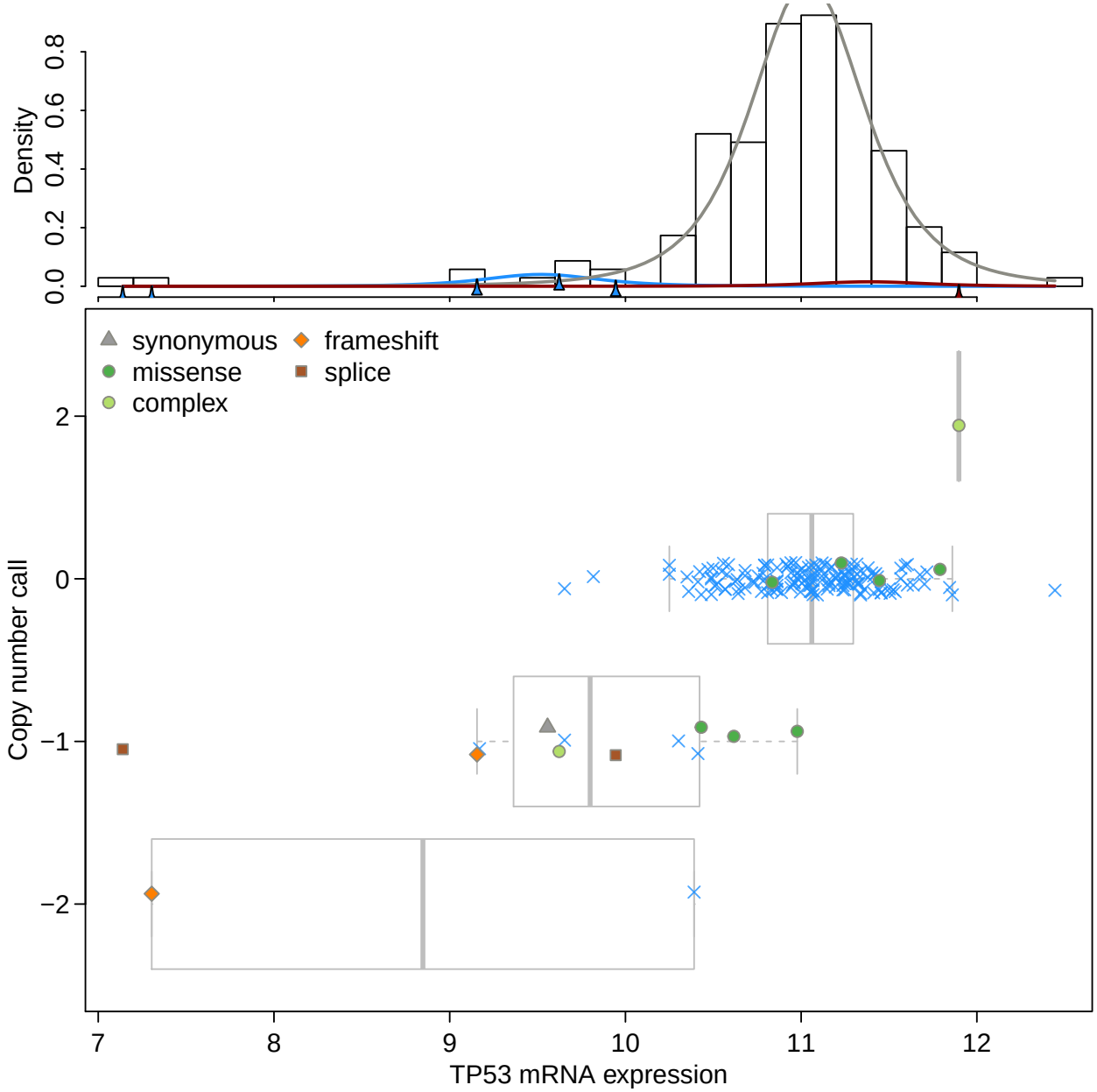
```
## $NPM1
##      MDM2      AKT1    HEXIM1      GRK5       SSB      NOP2     HMGA2
##         1         1         1         1         1         1         1
##      PPID HIST2H2BE     BAALC      AFF1      TET2     THOC7     HOXA7
##         1         1         1         1         1         1         1
##     KPNB1        PC      SMC4      IDH1       KIT      CD34    STAT5B
##         1         1         1         1         1         1         1
##       MN1
##         1
##
## $RUNX1
##  SUV39H1     ETS1    SMAD4    RUNX3   IGFBP3     PIM1    CEBPA     TLE1
##        1        1        1        1        1        1        1        1
##    CCND1    SMAD3  SMARCA4       AR    TCF12    SMAD1     LMO2     CD34
##        1        1        1        1        1        1        1        1
##   NT5DC3     HRAS   ZNF687    CDC73 NOTCH2NL   YTHDF2     PBX1     JAK2
##        1        1        1        1        1        1        1        1
##    FHOD1    ZFPM1     ABL1    ZFP64     GFI1    BAALC
##        1        1        1        1        1        1
```

Cis-analysis

We first analyze the cis-effects of loss-of-function mutations (frameshift, nonsense and splice-site mutations) on gene expression.

```
weight    = EstimateExpression(expr)

# Impute missing values
expr      = ImputeKnn(expr)
cna.logr  = ImputeKnn(cna.logr)

# Quantile-Normalization
expr.quantile = QuantileNorm(expr)
```

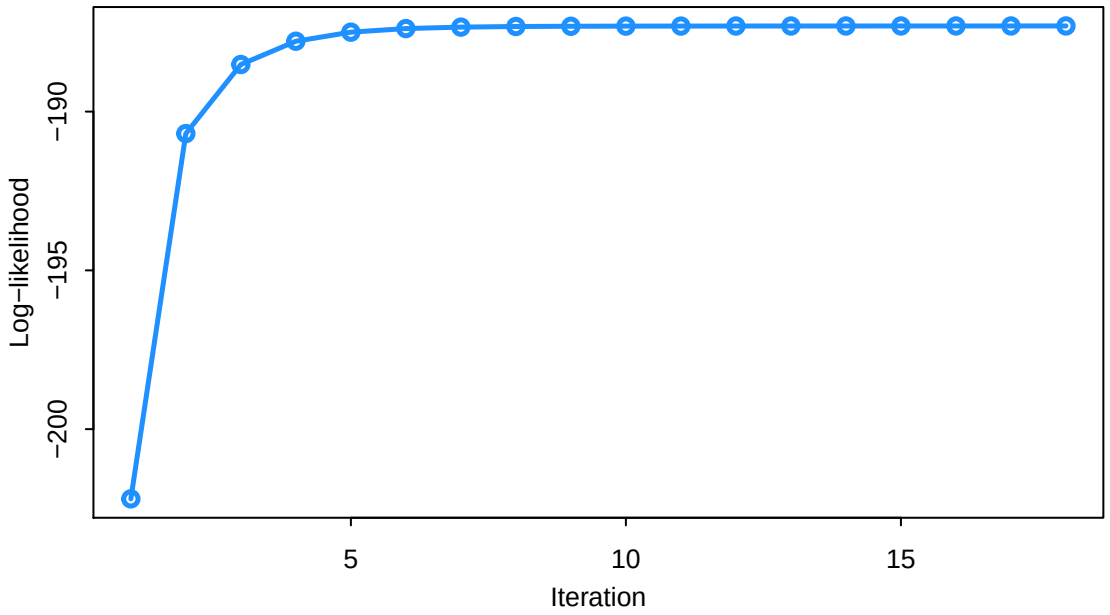
```
#=============================================================================================
## Get the conditional distritions P(Y|G)
#
# We first show TP53 mutations, expression, and copy number alterations
tmp  = GetExpressionDistribution(expr=expr.quantile, mut=mut, cna.call=cna.call,
                                 gene="TP53", show.plot=TRUE)
```




```
expr.dis.quantile  = GetExpressionDistribution(expr=expr.quantile, mut=mut)
```

```
#=============================================================================
## Filtering not expressed genes, and only analyzing loss-of-function
## Mutations
##
id = weight[mut[, "hgnc_symbol"]] > 0.9 &
     (mut[, "variant_type"] %in% c("FRAMESHIFT", "NONSENSE", "SPLICE"))
id = id & !is.na(id)
mut.filt = mut[id, ]


#=============================================================================
init = SetXseqPrior(expr.dis = expr.dis.quantile,
```

```
                mut      = mut.filt,
                mut.type = "loss",
                cis      = TRUE)

# parameter constraints in EM-iterations
constraint  = list(equal.fg=FALSE)

model.cis = InitXseqModel(mut            = mut.filt,
                          expr           = expr.quantile,
                          expr.dis       = expr.dis.quantile,
                          cpd            = init$cpd,
                          cis            = TRUE,
                          prior          = init$prior)

model.cis.em = LearnXseqParameter(model      = model.cis,
                                  constraint = constraint,
                                  iter.max   = 50,
                                  threshold  = 1e-6)
```



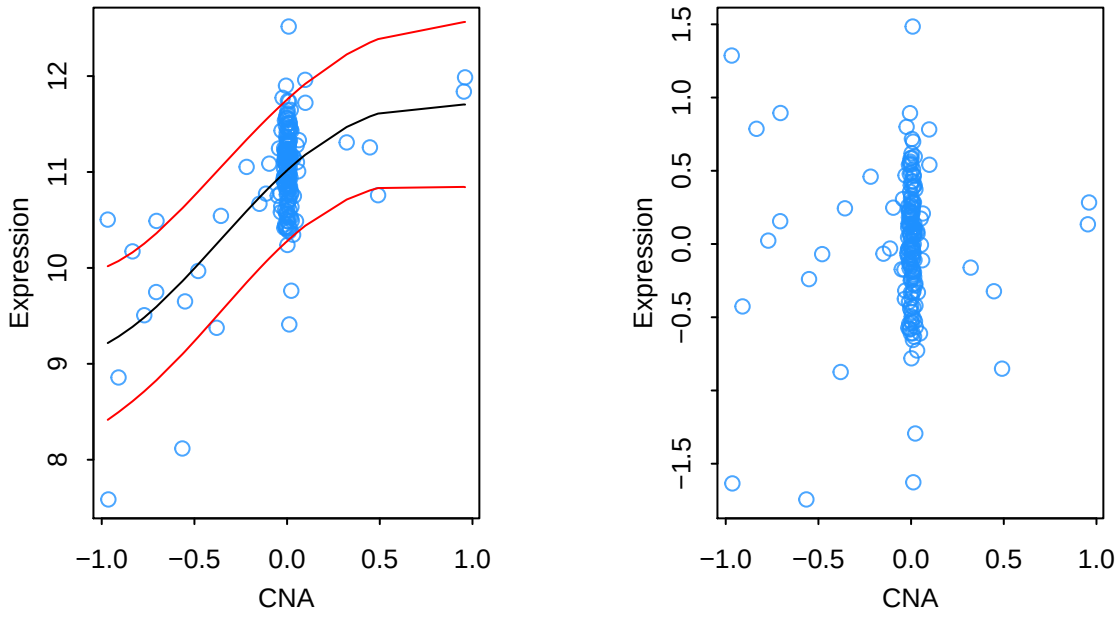

```
xseq.pred = ConvertXseqOutput(model.cis.em$posterior)
xseq.pred[1:20,]
```

```
##              sample hgnc_symbol      P(F)      P(D)
## 67  TCGA-AB-2820-03        TP53 0.9639755 0.9905104
## 68  TCGA-AB-2857-03        TP53 0.9613231 0.9905104
## 69  TCGA-AB-2860-03        TP53 0.9818841 0.9905104
## 70  TCGA-AB-2868-03        TP53 0.9648281 0.9905104
## 71  TCGA-AB-2908-03        TP53 0.9796830 0.9905104
## 72  TCGA-AB-2938-03        TP53 0.7893094 0.9905104
## 113 TCGA-AB-2871-03       STAG2 0.9749255 0.9846371
## 114 TCGA-AB-2913-03       STAG2 0.7286295 0.9846371
## 115 TCGA-AB-2964-03       STAG2 0.9779552 0.9846371
## 116 TCGA-AB-2972-03       STAG2 0.9769733 0.9846371
```

```
## 117 TCGA-AB-2978-03       STAG2 0.9789665 0.9846371
## 62  TCGA-AB-2818-03       RAD21 0.8466841 0.9823520
## 63  TCGA-AB-2886-03       RAD21 0.9700700 0.9823520
## 64  TCGA-AB-2967-03       RAD21 0.9708465 0.9823520
## 65  TCGA-AB-2975-03       RAD21 0.9654264 0.9823520
## 66  TCGA-AB-2986-03       RAD21 0.9684474 0.9823520
## 118 TCGA-AB-2900-03       SMC1A 0.8430609 0.6738602
## 9   TCGA-AB-2807-03      POLR2E 0.7352240 0.5944941
## 108 TCGA-AB-2851-03        SMC3 0.7756835 0.5110214
## 109 TCGA-AB-2950-03        SMC3 0.4095276 0.5110214
```

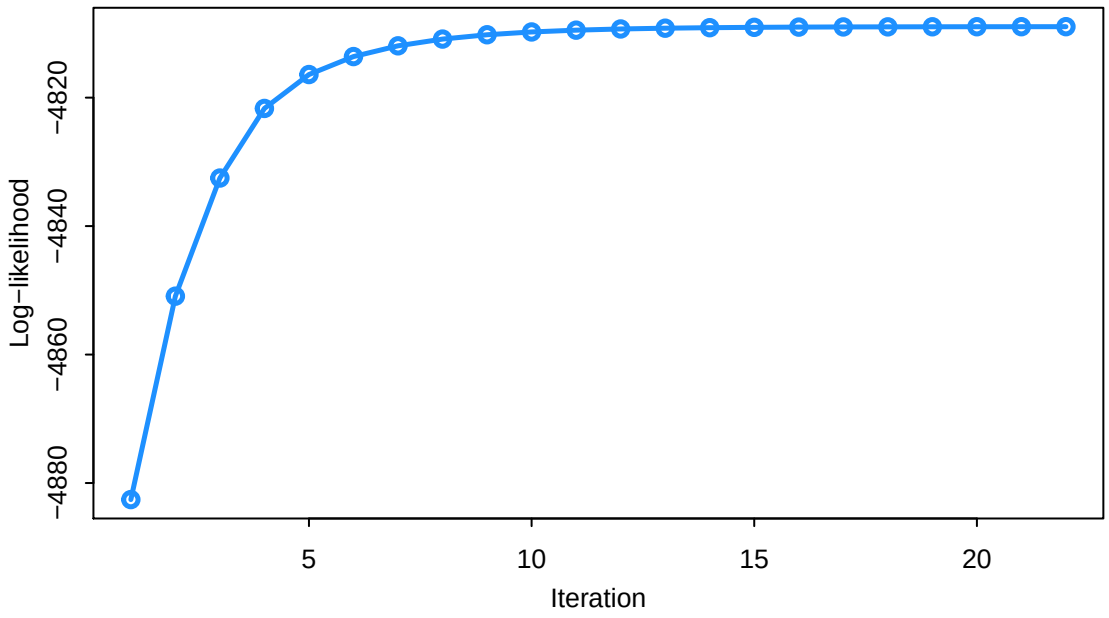
Trans-analysis

```
#=============================================================================================
## Remove the cis-effects of copy number alterations on gene expression
#
# We first show an example: PTEN copy number alterations and expression in AML
tmp = NormExpr(cna.logr=cna.logr, expr=expr, gene="TP53", show.plot=TRUE)
```




```
expr.norm = NormExpr(cna.logr=cna.logr, expr=expr)
expr.norm.quantile = QuantileNorm(expr.norm)

#=============================================================================================
## Get the conditional distritions P(Y|G),
#
expr.dis.norm.quantile  = GetExpressionDistribution(expr=expr.norm.quantile,
                                                    mut=mut)


#=============================================================================================
##
```

```
## Filtering not expressed genes
##

id = weight[mut[, "hgnc_symbol"]] > 0.9
id = id & !is.na(id)
mut.filt = mut[id, ]


#=============================================================================================
# Filter the network by only keeping the top-50 genes,
# with connection strength no less than 0.4
net = sapply(net, function(z) {
  z = z[z >= 0.4]
  if (length(z) > 50) {
    z = z[1:50]
  }
  if (length(z) < 5) {
    z = NULL
  }
  return (z)
})

init = SetXseqPrior(expr.dis = expr.dis.norm.quantile,
                net      = net,
                mut      = mut.filt,
                mut.type = "both",
                cis      = FALSE)

# parameter constraints in EM-iterations
constraint  = list(equal.fg=TRUE, baseline=init$baseline)

model.trans = InitXseqModel(mut        = mut.filt,
                            expr       = expr.norm.quantile,
                            net        = net,
                            expr.dis   = expr.dis.norm.quantile,
                            cpd        = init$cpd,
                            cis        = FALSE,
                            prior      = init$prior)

## EM algorithm for parameter estimations
model.trans.em = LearnXseqParameter(model      = model.trans,
                                    constraint = constraint,
                                    iter.max   = 50,
                                    threshold  = 1e-6)
```

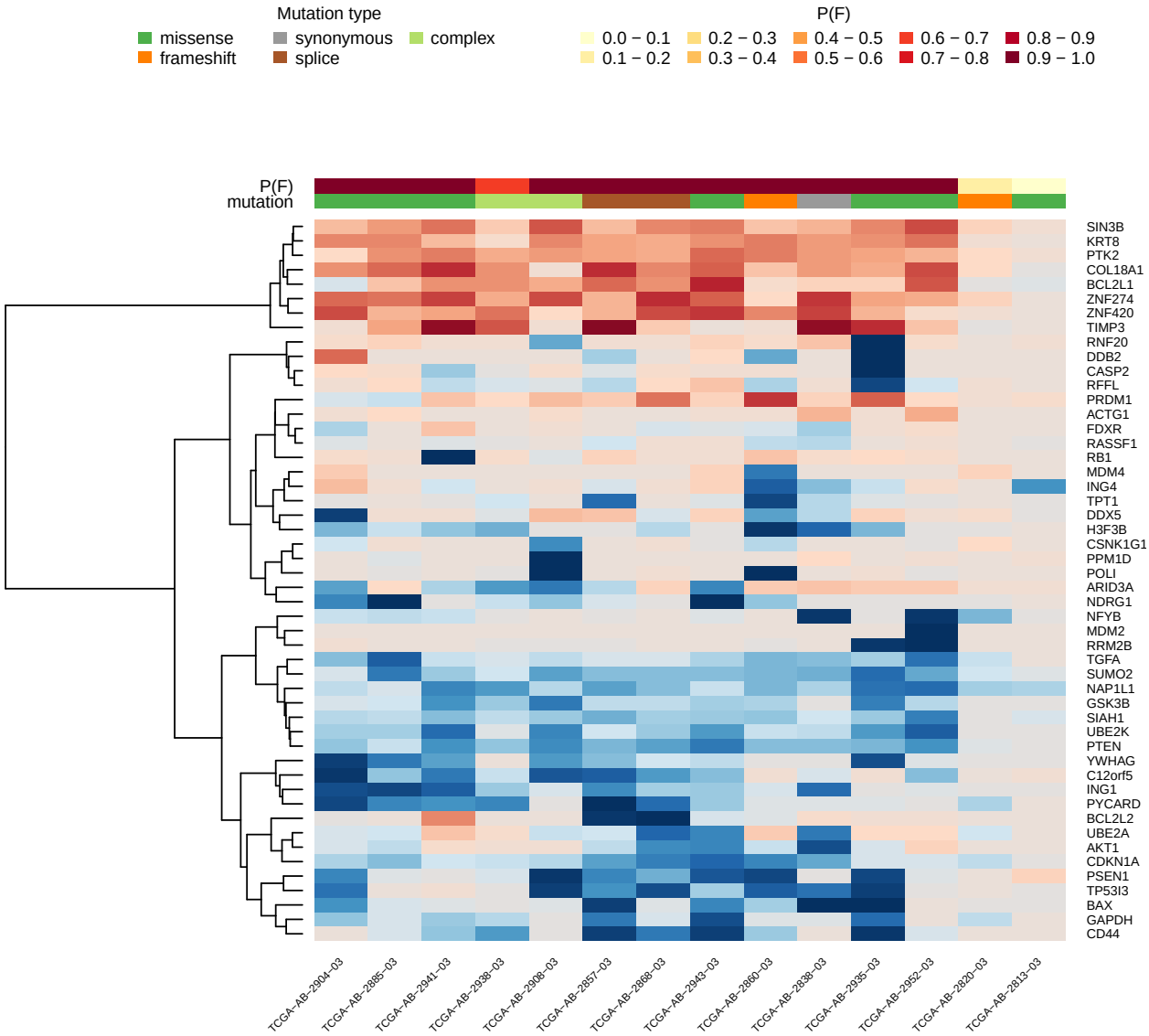



```
#=============================================================================================
# Reformat output

xseq.pred = ConvertXseqOutput(model.trans.em$posterior)
xseq.pred[1:20, ]
```

```
##             sample hgnc_symbol      P(F) P(D)
## 49 TCGA-AB-2810-03        NPM1 0.6150921    1
## 50 TCGA-AB-2811-03        NPM1 0.7559845    1
## 51 TCGA-AB-2812-03        NPM1 0.9998402    1
## 52 TCGA-AB-2816-03        NPM1 0.7741887    1
## 53 TCGA-AB-2818-03        NPM1 0.9989033    1
## 54 TCGA-AB-2824-03        NPM1 0.9493704    1
## 55 TCGA-AB-2825-03        NPM1 0.9901913    1
## 56 TCGA-AB-2826-03        NPM1 0.8165706    1
## 57 TCGA-AB-2835-03        NPM1 0.8168570    1
## 58 TCGA-AB-2836-03        NPM1 0.7120723    1
## 59 TCGA-AB-2837-03        NPM1 0.8694290    1
## 60 TCGA-AB-2839-03        NPM1 0.9338839    1
## 61 TCGA-AB-2848-03        NPM1 0.9450092    1
## 62 TCGA-AB-2853-03        NPM1 0.9999715    1
## 63 TCGA-AB-2859-03        NPM1 0.7302909    1
## 64 TCGA-AB-2861-03        NPM1 0.9952025    1
## 65 TCGA-AB-2869-03        NPM1 0.8525880    1
## 66 TCGA-AB-2871-03        NPM1 0.5290987    1
## 67 TCGA-AB-2877-03        NPM1 0.9738998    1
## 68 TCGA-AB-2879-03        NPM1 0.9609612    1
```

```
# We finally show the dysregulation probabilites of genes connected to TP53
tmp = PlotRegulationHeatmap(gene="TP53", posterior=model.trans.em$posterior, main="in_AML",
                    mut=mut, subtype=list(NULL), key=FALSE, dendrogram="row")
```

TP53_in_AML


Mutation type
missense
frameshift
synonymous
splice
complex
P(F)
0.0 − 0.1
0.1 − 0.2
0.2 − 0.3
0.3 − 0.4
0.4 − 0.5
0.5 − 0.6
0.6 − 0.7
0.7 − 0.8
0.8 − 0.9
0.9 − 1.0
P(F)
mutation
SIN3B
KRT8
PTK2
COL18A1
BCL2L1
ZNF274
ZNF420
TIMP3
RNF20
DDB2
CASP2
RFFL
PRDM1
ACTG1
FDXR
RASSF1
RB1
MDM4
ING4
TPT1
DDX5
H3F3B
CSNK1G1
PPM1D
POLI
ARID3A
NDRG1
NFYB
MDM2
RRM2B
TGFA
SUMO2
NAP1L1
GSK3B
SIAH1
UBE2K
PTEN
YWHAG
C12orf5
ING1
PYCARD
BCL2L2
UBE2A
AKT1
CDKN1A
PSEN1
TP53I3
BAX
GAPDH
CD44
TCGA-AB-2904-03
TCGA-AB-2885-03
TCGA-AB-2941-03
TCGA-AB-2938-03
TCGA-AB-2908-03
TCGA-AB-2857-03
TCGA-AB-2868-03
TCGA-AB-2943-03
TCGA-AB-2860-03
TCGA-AB-2838-03
TCGA-AB-2935-03
TCGA-AB-2952-03
TCGA-AB-2820-03
TCGA-AB-2813-03