

# splicing\_factors\_heatmap\_day15

2023-10-20

```
library(tidyr)
library(dplyr)
library(ComplexHeatmap)
library(clusterProfiler)
library(here)
here::i_am("R/03_splicing_factors_heatmap_day15.Rmd")
```

```
rpkm = read.delim(here("03limma", "beige_day15_rpkm_tmm_means.tab"))
head(rpkm)
```

```
##           Geneid Length gene_name
## 1 ENSG00000000003    4536   TSPAN6
## 2 ENSG00000000005    1476    TNMD
## 3 ENSG00000000419    1207    DPM1
## 4 ENSG00000000457    6883   SCYL3
## 5 ENSG00000000460    5970  C1orf112
## 6 ENSG00000000938    3382    FGR
##
##                                     description  gene_biotype
## 1                                     tetraspanin 6  protein_coding
## 2                                     tenomodulin    protein_coding
## 3 dolichyl-phosphate mannosyltransferase subunit 1, catalytic  protein_coding
## 4                                     SCY1 like pseudokinase 3  protein_coding
## 5                                     chromosome 1 open reading frame 112  protein_coding
## 6                                     FGR proto-oncogene, Src family tyrosine kinase  protein_coding
##  ensembl_gene_id_version subject1.beige subject1.white subject2.beige
## 1      ENSG00000000003.15      7.015370      5.0752183      12.5300941
## 2      ENSG00000000005.6      1.627583      0.3200113      30.6612441
## 3      ENSG00000000419.12     30.635853     33.4089794      32.3055705
## 4      ENSG00000000457.14      1.862880      1.4176901      2.3720554
## 5      ENSG00000000460.17      0.393713      0.4077405      0.4788014
## 6      ENSG00000000938.13      2.977751      0.7902128      4.6470874
##  subject2.white subject3.beige subject3.white subject4.beige subject4.white
## 1      6.4851444      9.7247952      6.2324136      9.3056202      9.0232992
## 2     30.2448972      2.7906896      0.4912616      2.3021246      0.6401144
## 3     29.1252400     34.1100563     34.7548040     32.7208903     34.6618668
## 4      1.5836657      2.1136964      1.5022350      2.1423301      1.7105704
## 5      0.3762281      0.4816479      0.4403470      0.4698593      0.5608791
## 6      0.8865716      7.1807572      0.9509210      5.8448505      0.6362375
##  subject5.beige subject5.white subject6.beige subject6.white
## 1     10.0681020      6.3019890     11.7900221      8.3815053
## 2      6.2119587      1.6856768      8.8600561      2.1400068
## 3     31.6549387     30.9930433     38.2724535     35.8339890
## 4      1.9104027      1.5130770      2.0849212      1.6548458
## 5      0.3557019      0.4699746      0.3751493      0.4361585
```

```
## 6          5.6321927      0.7380337      12.7832922      1.9632742
```

set up heatmap matrices

```
rpkm$gene_name[duplicated(rpkm$gene_name)]
```

```
## [1] "CD99"      "SLC25A6"   "GTPBP6"    "Y_RNA"     "Y_RNA"     "Y_RNA"
## [7] "Y_RNA"     "Y_RNA"     "Y_RNA"     "Y_RNA"     "BMS1P4"    "POLR2J4"
## [13] "MATR3"     "HSPA14"    "TBCE"      "POLR2J3"   "AHRR"      "C2orf27A"
```

```
rpkm = rpkm[!duplicated(rpkm$gene_name),]
rownames(rpkm) = rpkm$gene_name
rmat = rpkm[,grep("beige|white", colnames(rpkm))]
tail (rmat)
```

```
##          subject1.beige subject1.white subject2.beige subject2.white
## AC005618.4    0.19327565    0.26156522    0.27244232    0.21231920
## AL022318.5    0.05008334    0.06833633    0.08021512    0.15143889
## FAM106C       0.38080708    0.37914769    0.03539279    0.19205603
## AC010332.3    0.03458649    0.05052052    0.04345704    0.06817003
## CRIPAK        1.15433350    1.45605812    1.04181365    1.38704983
## AL109627.1    0.98793519    1.39978120    0.29364086    0.82971890
##          subject3.beige subject3.white subject4.beige subject4.white
## AC005618.4    0.193890828    0.209541955    0.187030458    0.19207329
## AL022318.5    0.027763170    0.054401627    0.007548142    0.02258869
## FAM106C       0.002384258    0.004406799    0.105184357    0.16605825
## AC010332.3    0.034930087    0.050085109    0.038787029    0.07133976
## CRIPAK        0.950599560    0.971724626    1.094710630    1.02932258
## AL109627.1    0.691190239    1.135051838    0.312630332    0.71429497
##          subject5.beige subject5.white subject6.beige subject6.white
## AC005618.4    0.15569489    0.13545189    0.17030631    0.27480056
## AL022318.5    0.07566827    0.08769766    0.00000000    0.01049002
## FAM106C       0.03577993    0.04848370    0.16900469    0.22934149
## AC010332.3    0.03473264    0.05364728    0.02968104    0.02685822
## CRIPAK        0.87594789    1.08185436    0.85676642    1.04604229
## AL109627.1    0.51394167    0.62222821    0.55453845    1.27463757
```

```
norm = t(scale(t(rmat)))
tail(norm)
```

```
##          subject1.beige subject1.white subject2.beige subject2.white
## AC005618.4   -0.25966141    1.2702403    1.51392165    0.1669741
## AL022318.5   -0.06778105    0.3536206    0.62786267    2.2721855
## FAM106C       1.76277678    1.7503367   -0.82673319    0.3477435
## AC010332.3   -0.70461973    0.4019181   -0.08860458    1.6275876
## CRIPAK        0.41349700    2.0663809   -0.20290031    1.6883452
## AL109627.1    0.58699210    1.7356168   -1.34937148    0.1457322
##          subject3.beige subject3.white subject4.beige subject4.white
## AC005618.4   -0.2458795    0.10475510   -0.39957348   -0.2865981
## AL022318.5   -0.5830804    0.03191402   -1.04977894   -0.7025421
## FAM106C      -1.0741924   -1.05902974   -0.30351815    0.1528427
## AC010332.3   -0.6807584    0.37168138   -0.41291324    1.8477095
```

```
## CRIPAK -0.7025821 -0.58685644 0.08687573 -0.2713279
## AL109627.1 -0.2406196 0.99729549 -1.29641047 -0.1761813
## subject5.beige subject5.white subject6.beige subject6.white
## AC005618.4 -1.1015891 -1.55509619 -0.7742471 1.5667537
## AL022318.5 0.5228910 0.80061006 -1.2240407 -0.9818606
## FAM106C -0.8238309 -0.72859298 0.1749316 0.6272660
## AC010332.3 -0.6944700 0.61905647 -1.0452781 -1.2413090
## CRIPAK -1.1115329 0.01644752 -1.2166113 -0.1797353
## AL109627.1 -0.7349599 -0.43295240 -0.6217368 1.3865954
```

## Get splicing factors

```
molsig <- clusterProfiler::read.gmt(here("annotations/msigdb.v2023.1.Hs.symbols.gmt"))
head(molsig); nrow(molsig)
```

```
## term gene
## 1 chr1p11 LINC02798
## 2 chr1p11 MTIF2P1
## 3 chr1p11 SRGAP2C
## 4 chr1p11 SRGAP2-AS1
## 5 chr1p11 LINC01691
## 6 chr1p11 NBPf26
```

```
## [1] 3961711
```

```
prefixes = c("HALLMARK", "KEGG", "REACTOME", "WP", "GOBP", "GOCC", "GOMF")
colnames(molsig) = c("term", "gene")
some.molsig = molsig[gsub("_.*", "", molsig$term) %in% prefixes,]
some.molsig$term = factor(some.molsig$term)
table(gsub("_.*", "", some.molsig$term))
```

```
##
## GOBP GOCC GOMF HALLMARK KEGG REACTOME WP
## 642656 98915 108833 7322 12796 92769 31635
```

```
reg_rnasplce = some.molsig$gene[grep("GOBP_REGULATION_OF_RNA_SPLICING", some.molsig$term)]
head(reg_rnasplce, n=50)
```

```
## [1] "PQBP1" "RBM12" "MBNL2" "RBM7" "RBM5" "SRRM1" "SF3B4"
## [8] "SAP18" "PRMT5" "TADA3" "DDX17" "TAF6L" "KHDRBS3" "KHDRBS1"
## [15] "CELF1" "CELF2" "SRSF10" "RNPS1" "FASTK" "HNRNPAO" "CELF3"
## [22] "SGF29" "AHNAK2" "U2AF2" "CIRBP" "TADA1" "CLK1" "CLK2"
## [29] "CLK3" "CLNS1A" "SRSF12" "RBF3X" "RBM1F" "DDX5" "DYRK1A"
## [36] "KHDRBS2" "ERN1" "RBM24" "PUF60" "HABP4" "SNW1" "SETX"
## [43] "RRP1B" "JMD6" "FMR1" "USP22" "AFF2" "SF3B3" "RBF3X2"
## [50] "STH"
```

```
summary(reg_rnasplce %in% rpkms$gene_name) #24 unexpressed
```

```
##      Mode   FALSE    TRUE
## logical      24     157
```

regulation of RNA splicing looks like a decent list; but perhaps the splicing paper have some more specific/curated checking ones ... Castella shows reactome mRNA splicing; 212 genes And then checks 47 “representative” spliceaid genes; though they list 71 proteins in the paper abstract. the site is incredibly slow, <http://www.introni.it/splicing.html> Tissue specific search tool also exists: [http://193.206.120.249/splicing\\_tissue.html](http://193.206.120.249/splicing_tissue.html)

```
splicing_proteins = read.delim(here("annotations", "SpliceAidF_Table1.csv"), sep = ";")
head(splicing_proteins)
```

```
##      Splicing.factor Binding.sites Conditional.binding.sites No.binding.sites
## 1          9G8          70              1              29
## 2         CUG-BP1          42              3              32
## 3         DAZAP1          12              0               3
## 4          ESRP1           1              0               1
## 5          ESRP2           1              0               1
## 6          ETR-3          31              4              36
```

```
splicing_proteins$no_punct = gsub("[ /]", "", splicing_proteins$Splicing.factor)
splicing_proteins$no_punct = gsub("Nova-", "NOVA", splicing_proteins$no_punct)
splicing_proteins
```

```
##      Splicing.factor Binding.sites Conditional.binding.sites No.binding.sites
## 1          9G8          70              1              29
## 2         CUG-BP1          42              3              32
## 3         DAZAP1          12              0               3
## 4          ESRP1           1              0               1
## 5          ESRP2           1              0               1
## 6          ETR-3          31              4              36
## 7          FMRP          43              4               4
## 8         Fox-1          12              0               2
## 9         Fox-2          13              0               3
## 10         hnRNP A0           1              0               0
## 11         hnRNP A1         143             17              39
## 12         hnRNP A2/B1        42              1               8
## 13         hnRNP A3           2              0               1
## 14         hnRNP C          21              7              13
## 15         hnRNP C1          11              2              19
## 16         hnRNP C2          10              0              16
## 17         hnRNP D          29              2              14
## 18         hnRNP D0           1              0               0
## 19         hnRNP DL          34              0               0
## 20         hnRNP E1          43             17              14
## 21         hnRNP E2          39             13              23
## 22         hnRNP F          67              8              26
## 23         hnRNP G           1              0               0
## 24         hnRNP H1          85              8              45
## 25         hnRNP H2         101              8              42
## 26         hnRNP H3          60              8              44
## 27         hnRNP I (PTB)     129             13              53
```

## 28	hnRNP J	1	0	12
## 29	hnRNP K	58	15	13
## 30	hnRNP L	172	4	9
## 31	hnRNP LL	13	0	2
## 32	hnRNP M	1	0	3
## 33	hnRNP P (TLS)	16	4	8
## 34	hnRNP Q	10	0	7
## 35	hnRNP U	19	3	0
## 36	HTra2?	7	0	3
## 37	HTra2?1	20	1	14
## 38	HuB	44	0	1
## 39	HuC	2	0	2
## 40	HuD	51	6	5
## 41	HuR	72	25	26
## 42	KSRP	22	2	7
## 43	MBNL1	92	11	34
## 44	Nova-1	25	4	18
## 45	Nova-2	12	4	9
## 46	nPTB	3	0	1
## 47	PSF	32	0	7
## 48	QKI	1	0	0
## 49	RBM25	1	0	1
## 50	RBM4	8	0	2
## 51	RBM5	7	0	2
## 52	Sam68	16	0	5
## 53	SAP155	1	0	0
## 54	SC35	172	5	47
## 55	SF1	24	1	5
## 56	SF2/ASF	248	15	52
## 57	SLM-1	1	0	0
## 58	SLM-2	6	0	0
## 59	SRm160	1	0	0
## 60	SRp20	74	0	23
## 61	SRp30c	25	9	6
## 62	SRp38	10	0	0
## 63	SRp40	68	7	27
## 64	SRp54	1	0	0
## 65	SRp55	64	7	24
## 66	SRp75	8	0	18
## 67	TDP43	22	1	8
## 68	TIA-1	39	2	7
## 69	TIAL1	37	2	2
## 70	YB-1	21	1	14
## 71	ZRANB2	19	0	4
## 72	Total	2590	245	896
##	no_punct			
## 1	9G8			
## 2	CUG-BP1			
## 3	DAZAP1			
## 4	ESRP1			
## 5	ESRP2			
## 6	ETR-3			
## 7	FMRP			
## 8	Fox-1			

```

## 9      Fox-2
## 10     hnRNPA0
## 11     hnRNPA1
## 12     hnRNPA2B1
## 13     hnRNPA3
## 14     hnRNPC
## 15     hnRNPC1
## 16     hnRNPC2
## 17     hnRNPD
## 18     hnRNPD0
## 19     hnRNPD1
## 20     hnRNPE1
## 21     hnRNPE2
## 22     hnRNPF
## 23     hnRNPG
## 24     hnRNPH1
## 25     hnRNPH2
## 26     hnRNPH3
## 27     hnRNPI (PTB)
## 28     hnRNPI
## 29     hnRNPK
## 30     hnRNPL
## 31     hnRNPLL
## 32     hnRNPM
## 33     hnRNPP (TLS)
## 34     hnRNPP
## 35     hnRNPU
## 36     HTra2?
## 37     HTra2?1
## 38     HuB
## 39     HuC
## 40     HuD
## 41     HuR
## 42     KSRP
## 43     MBLN1
## 44     NOVA1
## 45     NOVA2
## 46     nPTB
## 47     PSF
## 48     QKI
## 49     RBM25
## 50     RBM4
## 51     RBM5
## 52     Sam68
## 53     SAP155
## 54     SC35
## 55     SF1
## 56     SF2ASF
## 57     SLM-1
## 58     SLM-2
## 59     SRm160
## 60     SRp20
## 61     SRp30c
## 62     SRp38

```

```
## 63      SRp40
## 64      SRp54
## 65      SRp55
## 66      SRp75
## 67      TDP43
## 68      TIA-1
## 69      TIAL1
## 70      YB-1
## 71      ZRANB2
## 72      Total
```

```
splicing_genes = HGNCHELPER::checkGeneSymbols(splicing_proteins$no_punct)
```

```
## Maps last updated on: Thu Oct 24 12:31:05 2019
```

```
## Warning in HGNCHELPER::checkGeneSymbols(splicing_proteins$no_punct): Human gene
## symbols should be all upper-case except for the 'orf' in open reading frames.
## The case of some letters was corrected.
```

```
## Warning in HGNCHELPER::checkGeneSymbols(splicing_proteins$no_punct): x contains
## non-approved gene symbols
```

```
splicing_genes$gene_name = splicing_genes$Suggested.Symbol
splicing_genes$gene_name[splicing_genes$x == "CUG-BP1"] = "CELF1" #genecards
splicing_genes$gene_name[splicing_genes$x == "hnRNPE1"] = "PCBP1" #genecards
splicing_genes$gene_name[splicing_genes$x == "hnRNPI(PTB)"] = "PTBP1" #genecards
splicing_genes$gene_name[splicing_genes$x == "hnRNPP(TLS)"] = "FUS" #genecards
splicing_genes$gene_name[splicing_genes$x == "HTra2?"] = "TRA2A" #genecards
splicing_genes$gene_name[splicing_genes$x == "HTra2?1"] = "TRA2B" #genecards
splicing_genes$gene_name[splicing_genes$x == "PSF"] = "SFPQ" #genecards to check which
splicing_genes$gene_name[splicing_genes$x == "SF2ASF"] = "SRSF1"
splicing_genes$gene_name[splicing_genes$x == "TDP43"] = "TARDBP" #genecards
splicing_genes = separate_rows(splicing_genes, gene_name, sep=" /// ")
splicing_genes
```

```
## # A tibble: 73 x 4
##       x      Approved Suggested.Symbol gene_name
##   <chr>   <lgl>      <chr>      <chr>
## 1 9G8     FALSE     SLU7 /// SRSF7  SLU7
## 2 9G8     FALSE     SLU7 /// SRSF7  SRSF7
## 3 CUG-BP1 FALSE     <NA>         CELF1
## 4 DAZAP1  TRUE      DAZAP1       DAZAP1
## 5 ESRP1   TRUE      ESRP1        ESRP1
## 6 ESRP2   TRUE      ESRP2        ESRP2
## 7 ETR-3   FALSE     CELF2        CELF2
## 8 FMRP    FALSE     FMR1         FMR1
## 9 Fox-1   FALSE     RBFOX1       RBFOX1
## 10 Fox-2  FALSE     RBFOX2       RBFOX2
## # i 63 more rows
```

9G8, both are possible, separate rows

C1/c2 here are just one gene by the looks, which is already included, same with hnrnpd0  
 couldn't find any mention of hnrnpJ, all that came up was k.  
 hnRNPP - aka TLS, aka FUS, aka hnrnpP-P2 the text format doesn't like the alpha and beta  
 SF2/asf= SRSF1

```
spliceaid = splicing_genes$gene_name[!is.na(splicing_genes$gene_name)]
length(spliceaid)
```

```
## [1] 68
```

```
summary(spliceaid %in% rownames(norm)) #not all are expressed
```

```
##      Mode   FALSE    TRUE
## logical      8      60
```

```
spliceaid[!spliceaid %in% rownames(norm)]
```

```
## [1] "ESRP1" "ESRP2" "RBFOX1" "ELAVL2" "ELAVL3" "ELAVL4" "NOVA2"
## [8] "KHDRBS2"
```

```
#elavl2 for e.g. has ensemblid ENSG00000107105
summary("ENSG00000107105" == rpkm$Geneid)
```

```
##      Mode   FALSE
## logical  18043
```

```
summary("ENSG00000104967" == rpkm$Geneid) #NOVA2 not expressed
```

```
##      Mode   FALSE
## logical  18043
```

```
summary("ENSG00000139910" == rpkm$Geneid) #NOVA1 is expressed.
```

```
##      Mode   FALSE    TRUE
## logical  18042      1
```

## Check DE

```
sig = read.delim(here("03limma","any_and_all_donor_DGE.tsv"))
colnames(sig)
```

```
## [1] "Geneid"          "gene_name"        "description"
## [4] "Length"          "gene_biotype"     "logFC.s4"
## [7] "logFC.s3"        "logFC.s2"         "logFC.s1"
## [10] "logFC.s6"        "logFC.s5"         "AveExpr"
## [13] "F"               "all.donors.P.Value" "all.donors.adj.P.Val"
```



```
## [16] "all.donors.AvelogFC" "P.Value.s3" "adj.P.Val.s3"
## [19] "AveExpr.s3" "P.Value.s4" "adj.P.Val.s4"
## [22] "AveExpr.s4" "P.Value.s2" "adj.P.Val.s2"
## [25] "AveExpr.s2" "P.Value.s1" "adj.P.Val.s1"
## [28] "AveExpr.s1" "P.Value.s6" "adj.P.Val.s6"
## [31] "AveExpr.s6" "P.Value.s5" "adj.P.Val.s5"
## [34] "AveExpr.s5"
```

```
all_sig = sig$gene_name[rowSums(sig[startsWith(colnames(sig), "adj.P.Val.s")] < 0.05) == 6]
length(all_sig)
```

```
## [1] 853
```

```
any_sig = sig$gene_name[sig$all.donors.adj.P.Val < 0.01]
length(any_sig)
```

```
## [1] 7554
```

```
summary(reg_rnasplce %in% all_sig) #NONE are sig in all donors
```

```
## Mode FALSE
## logical 181
```

```
summary(reg_rnasplce %in% any_sig) #only 52 are significant in any donor
```

```
## Mode FALSE TRUE
## logical 129 52
```

```
52/157 #only 33% of expressed factors are DE in any donor
```

```
## [1] 0.3312102
```

```
summary(spliceaid %in% all_sig) #NONE are sig in all donors
```

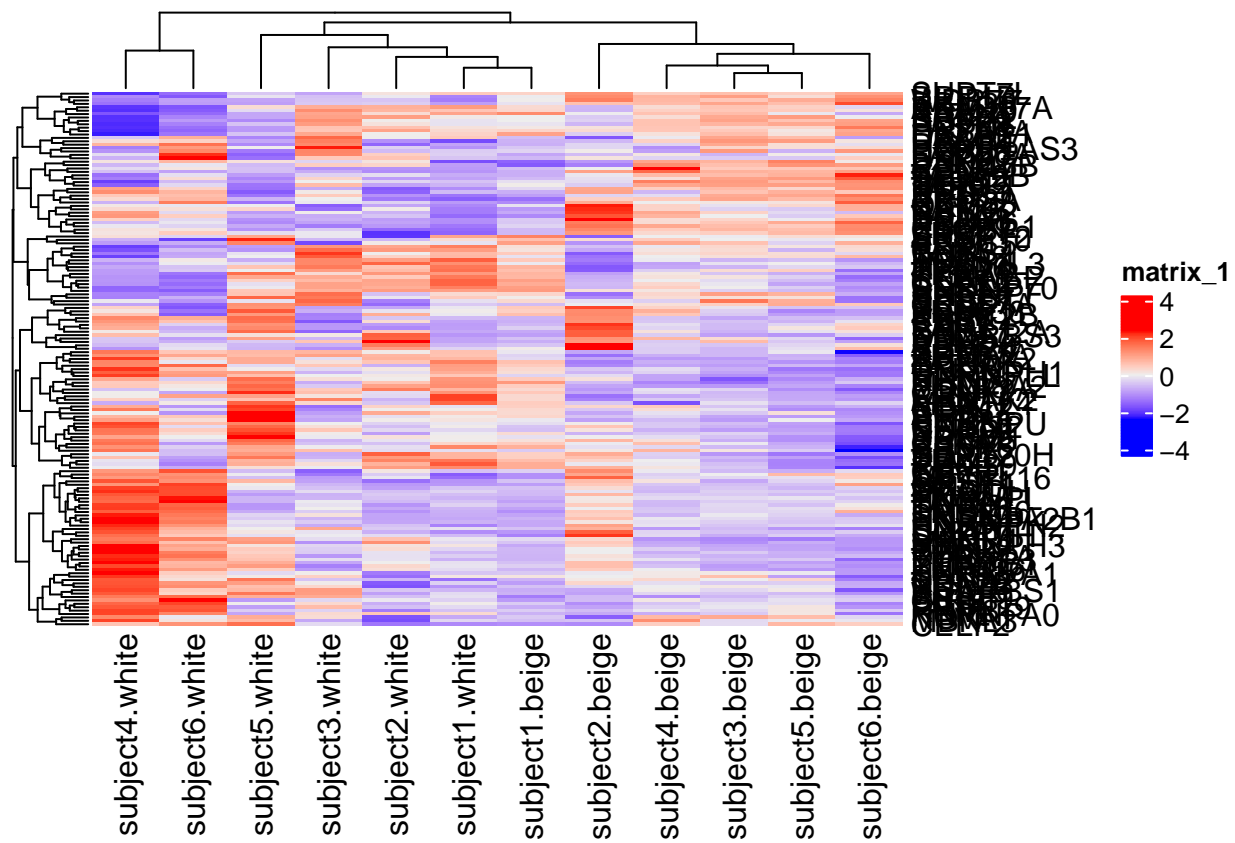
```
## Mode FALSE
## logical 68
```

```
summary(spliceaid %in% any_sig) #only 17 are significant in any donor
```

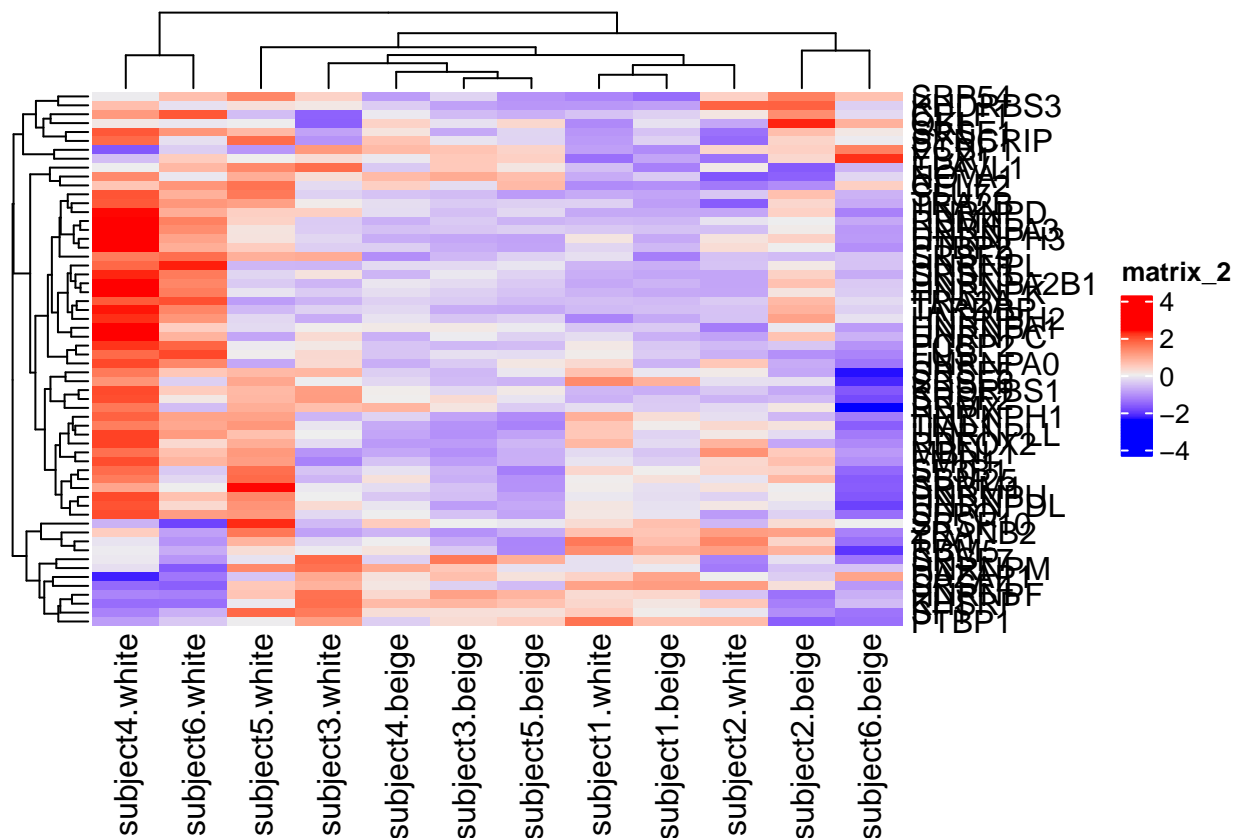
```
## Mode FALSE TRUE
## logical 51 17
```

## Heatmaps

```
Heatmap(norm[rownames(norm) %in% reg_rnasplce,])
```



```
Heatmap(norm[rownames(norm) %in% spliceaid,])
```



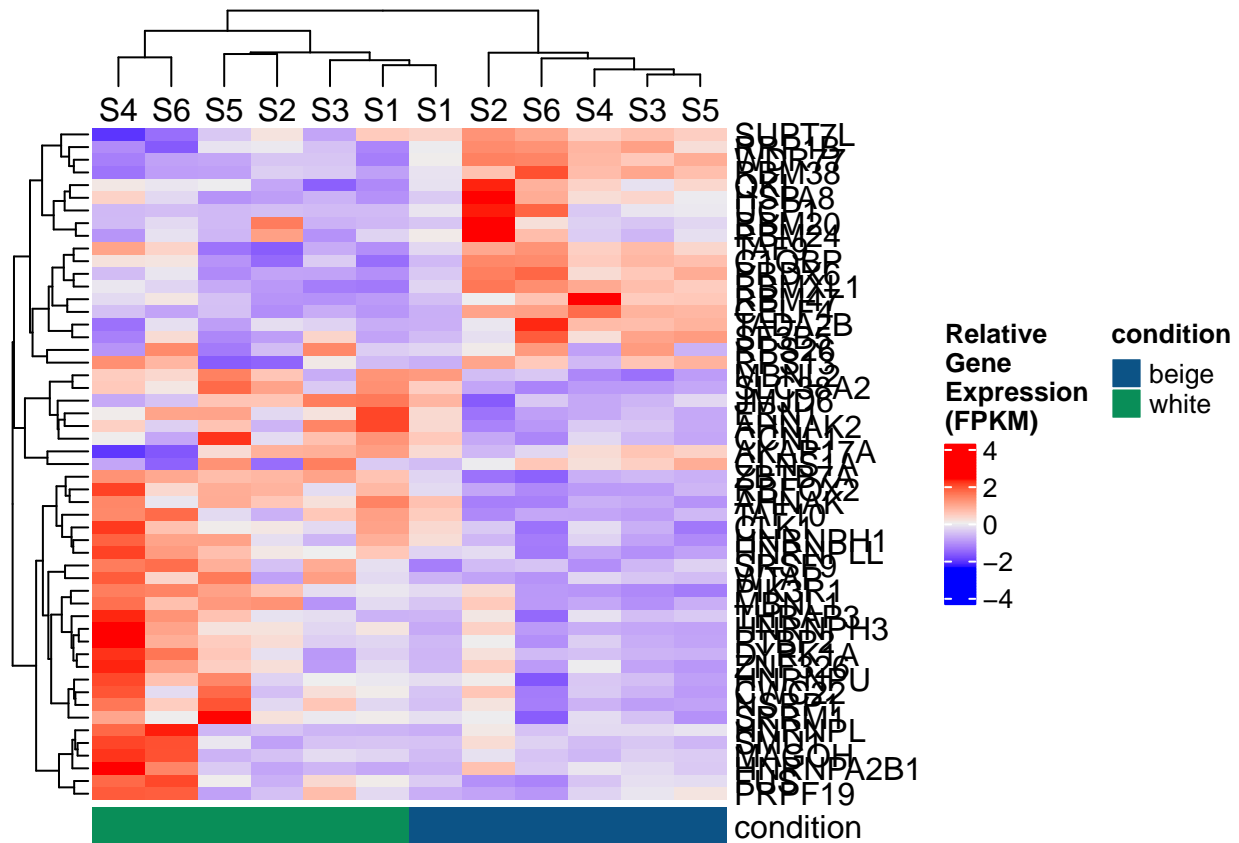
```
treat = gsub(".*\\."," ",colnames(norm))
treat
```

```
## [1] "beige" "white" "beige" "white" "beige" "white" "beige" "white" "beige"
## [10] "white" "beige" "white"
```

```
treatann = HeatmapAnnotation(condition=treat,
                             col=list(treat =c(white="#74B2CD",beige="#C1A486")))

donors = gsub("\\\\."," ",colnames(norm))
hm = Heatmap(norm[rownames(norm) %in% c(reg_rnasplce, "UCP1") &
                 rownames(norm) %in% any_sig,], name="Relative \nGene \nExpression \n(FPKM)",
             bottom_annotation = treatann, column_title_side = "top", ,
             column_labels = gsub("subject","S",donors), column_names_side = "top",
             column_names_rot=0, column_names_centered = T,
             height=5)

hm
```



```
pdf(here("R/plots", "reg_rnasplce_factors_any_sig.pdf"), width=5, height=7)
hm
dev.off
```

```
## function (which = dev.cur())
## {
##   if (which == 1)
##     stop("cannot shut down device 1 (the null device)")
##   .External(C_devoff, as.integer(which))
##   dev.cur()
## }
## <bytecode: 0x492d450>
## <environment: namespace:grDevices>
```

```
Heatmap(norm[rownames(norm) %in% c(spliceaid, "UCP1") &
      rownames(norm) %in% any_sig,])
```

```
sig[sig$gene_name == "QKI",]
```

```
##           Geneid gene_name           description Length
## 3380 ENSG00000112531      QKI QKI, KH domain containing RNA binding 17364
##           gene_biotype logFC.s4 logFC.s3 logFC.s2 logFC.s1 logFC.s6
## 3380 protein_coding -0.05491998 0.8504834 1.092032 0.4747053 0.2602345
##           logFC.s5 AveExpr           F all.donors.P.Value all.donors.adj.P.Val
```

```

## 3380 0.2000905 8.189493 11.50957      1.794058e-06      1.202765e-05
##      all.donors.AvelogFC    P.Value.s3 adj.P.Val.s3 AveExpr.s3 P.Value.s4
## 3380      0.4704376 6.981838e-05 0.0009419605    8.189493 0.8176884
##      adj.P.Val.s4 AveExpr.s4    P.Value.s2 adj.P.Val.s2 AveExpr.s2 P.Value.s1
## 3380      0.8836397    8.189493 1.549648e-06 2.533872e-05    8.189493 0.01385392
##      adj.P.Val.s1 AveExpr.s1 P.Value.s6 adj.P.Val.s6 AveExpr.s6 P.Value.s5
## 3380      0.1248653    8.189493 0.1906393    0.3173286    8.189493 0.297592
##      adj.P.Val.s5 AveExpr.s5
## 3380      0.4526537    8.189493

```