Unified classification and risk-stratification in Acute Myeloid Leukemia

Tutorial for Bayesian Dirichlet Process for finding molecular clusters :

This notebook is independent of the paper and can be used for clustering any dataset

In [8]:
```
library(hdp)
library(ggplot2)
library('ggrepel')
library(dplyr)

library('IRdisplay')
source('src/tools.R')
source('src/hdp_tools.R')    ## containing useful functions for HDP
library(gridExtra)
```

In [9]:
```
df_hdp1 <- read.table("data/aml_bdp1.tsv")

df_molecular <- read.table("data/aml_molecular_bdp.tsv")

genes_columns <-colnames(df_molecular)[grepl("^[[:upper:]]", colnames(df_molecular))]

cytos_columns <- colnames(df_molecular)[!grepl("^[[:upper:]]", colnames(df_molecular))]
```

BDP : A clustering tool to identify dominant molecular drivers

I) BDP1 : BDP algorithm applied on 2150 Patients and 153 genes and cytos

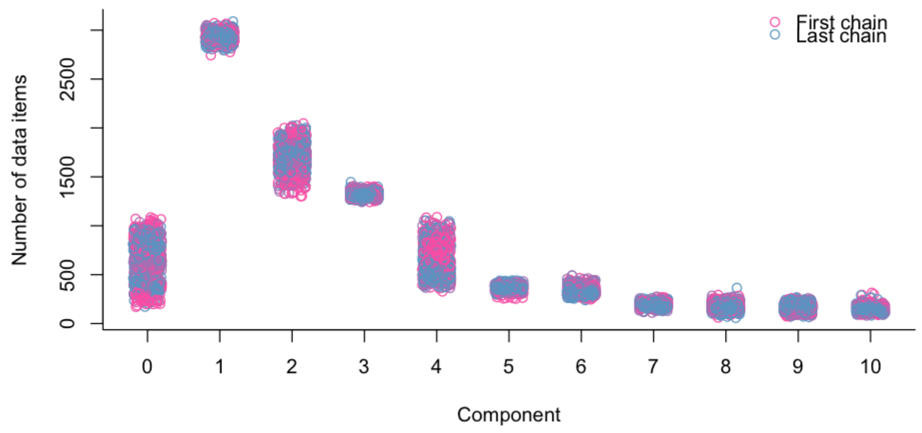
In [10]:
```
# HDP Parameters

data <- df_hdp1
posterior_samples <- 250
initial_clusters <- 17
burn <- 7000
chains <- 3
base_dist <- prepare_distributions(df_hdp1)$gaussian
aa <- 0.5
ab <-1.5
```

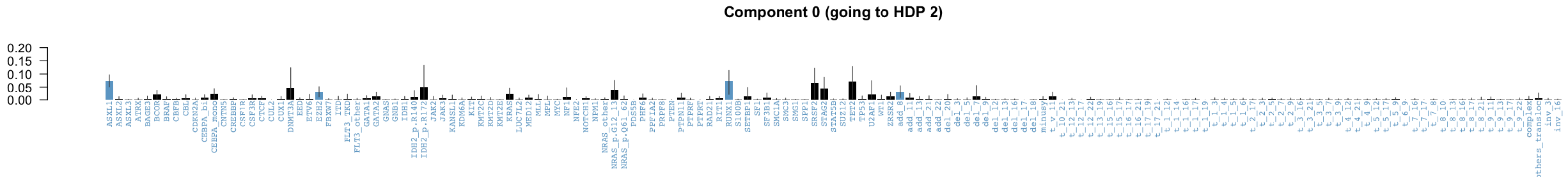
In [12]:
```
dd_predicted_10_components <- get_prediction_result_dataframe(multi_output_cluster_10, df_hdp1)
tab <- get_table(dd_predicted_10_components[,'predicted_component'])
tab


#plot components

plot_components_size(multi_output_cluster_10,8,4.5)
set_notebook_plot_size(22, 2.6)
plot_comp_distn(multi_output_cluster_10,
                cat_names   = colnames(df_hdp1),
                col         = "skyblue3",
                col_nonsig = "black",
                plot_title=c("Component 0 (going to HDP 2)","Component 1 (NPM1)","Component 2 (going
 to HDP 2)","Component 3 (TP53-Complex)","Component 4 (going to HDP 2)",
                             "Component 5 (t(8;21))","Component 6 (inv(16))","Component 7 (CEBPA_bi)"
,"Component 8 (t(15;17) & t(11))", "Component 9 (additions)","Component 10 (inv(3))")
                                         )
```

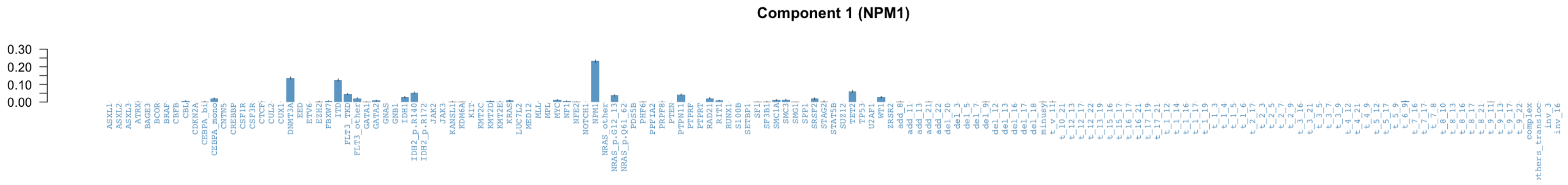
```
Number of components: 10
Number of NA rows   : 51
```

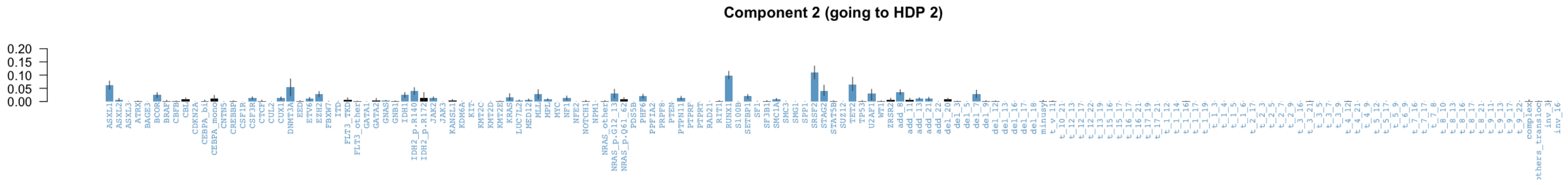
A data.frame: 13 × 3

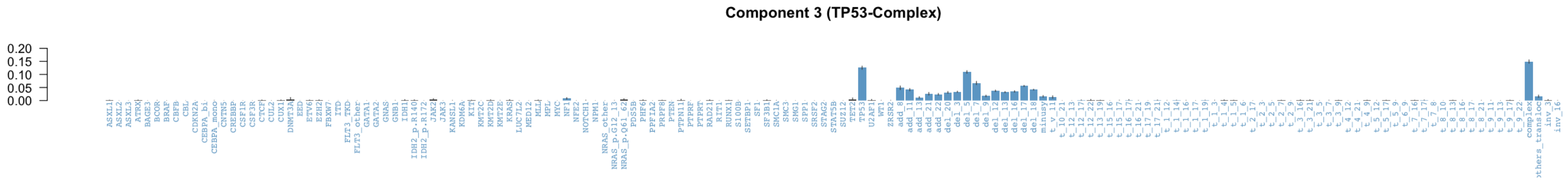
	values	count	freq
	<fct>	<int>	<chr>
2	1	833	38.7441860465116
3	2	418	19.4418604651163
4	3	229	10.6511627906977
5	4	128	5.95348837209302
6	5	114	5.30232558139535
7	6	114	5.30232558139535
1	0	96	4.46511627906977
9	8	61	2.83720930232558
12	NaN	51	2.37209302325581
8	7	50	2.32558139534884
11	10	29	1.34883720930233
10	9	27	1.25581395348837
13	-- total --	2150	100%


First chain
Last chain
Number of data items
Component
0
500
1500
2500
0 1 2 3 4 5 6 7 8 9 10

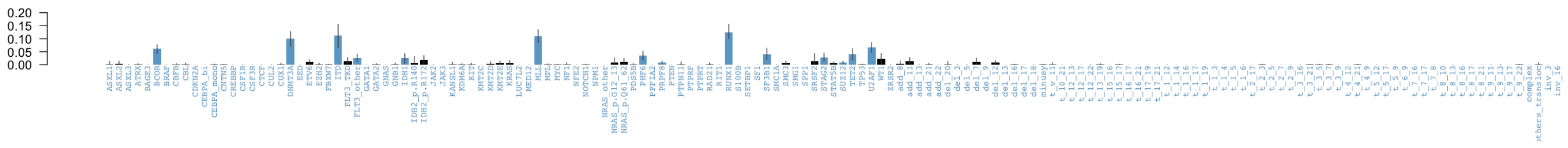

Component 0 (going to HDP 2)
0.20
0.15
0.10
0.05
0.00


Component 1 (NPM1)
0.30
0.20
0.10
0.00

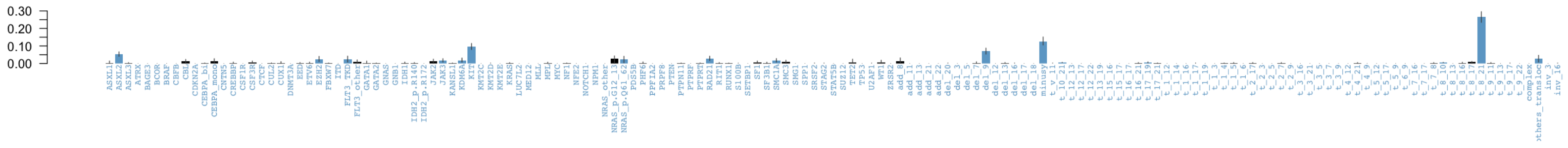

Component 2 (going to HDP 2)
0.20
0.15
0.10
0.05
0.00


Component 3 (TP53-Complex)
0.20
0.15
0.10
0.05
0.00

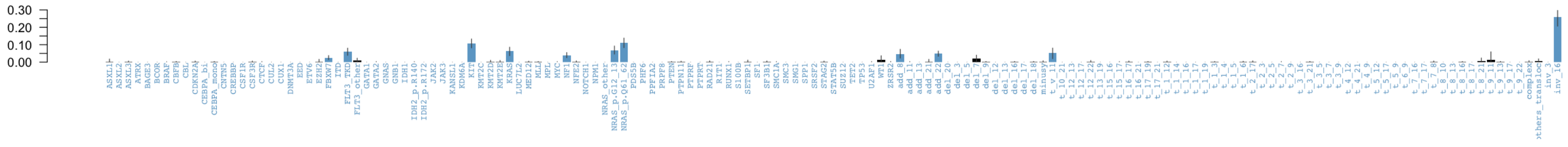
Component 4 (going to HDP 2)



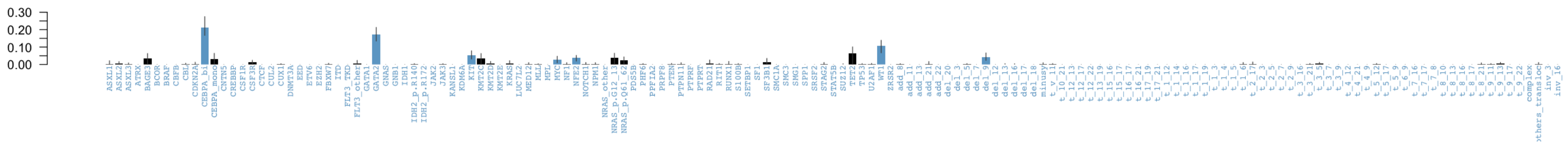
Component 5 (t(8;21))



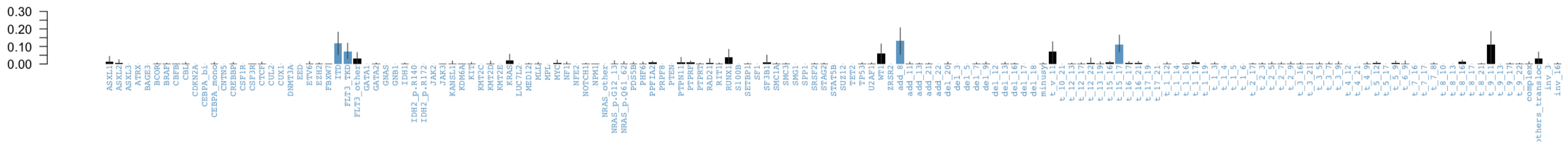
Component 6 (inv(16))



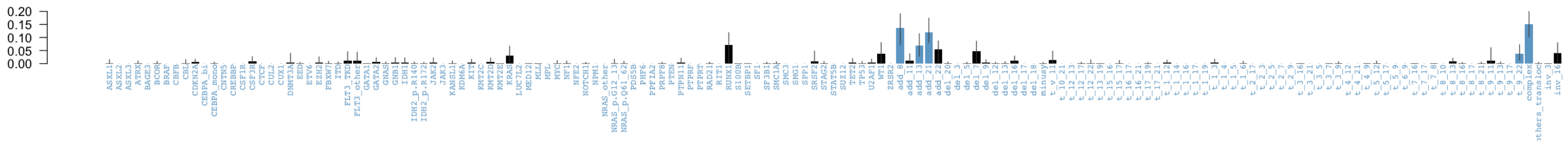
Component 7 (CEBPA_bi)

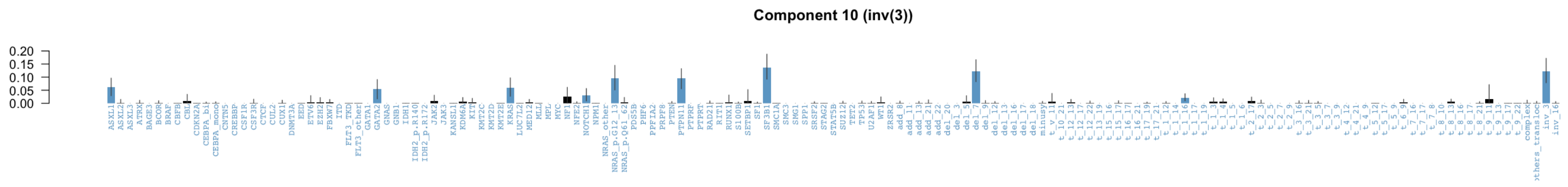


Component 8 (t(15;17) & t(11))



Component 9 (additions)



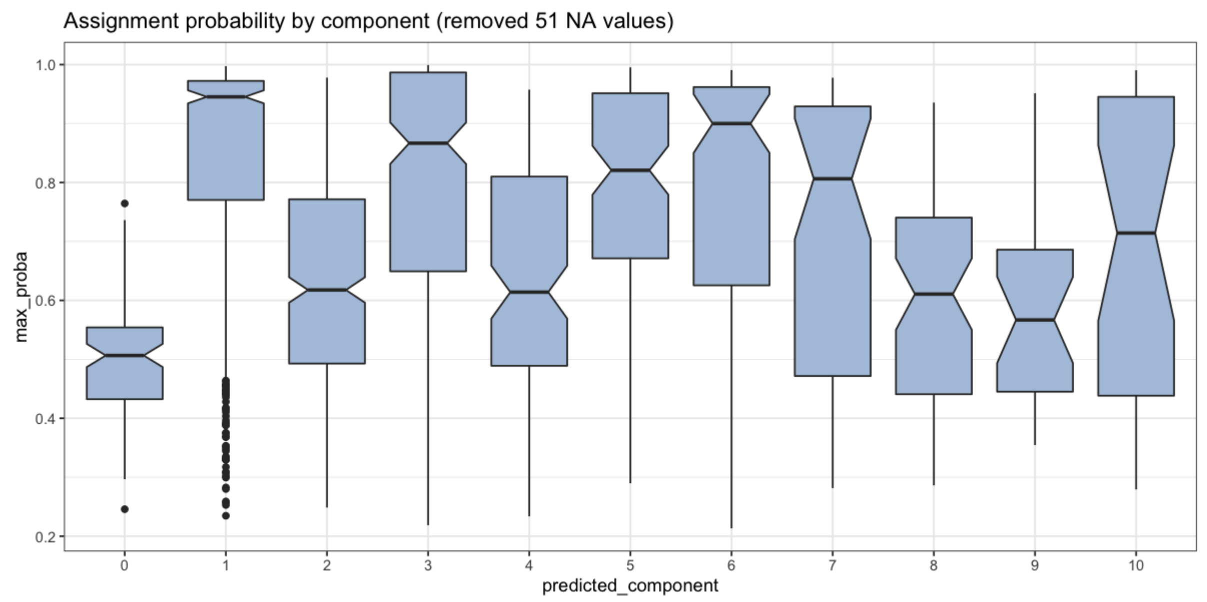

Component 10 (inv(3))
0.20
0.15
0.10
0.05
0.00
ASXL1
ASXL2
ASXL3
ATRX
BAGE3
BCOR
BRAF
CBFB
CBL
CDKN2A
CEBPA_bi
CEBPA_mono
CNTN5
CREBBP
CSF1R
CSF3R
CTCF
CUL2
CUX1
DNMT3A
EED
ETV6
EZH2
FBXW7
ITD
FLT3_TKD
FLT3_other
GATA1
GATA2
GNAS
GNB1
IDH1
IDH2_p.R140
IDH2_p.R172
JAK2
JAK3
KANSL1
KDM6A
KIT
KMT2C
KMT2D
KMT2E
KRAS
LUC7L2
MED12
MLL
MPL
MYC
NF1
NFE2
NOTCH1
NPM1
NRAS_other
NRAS_p.G12_13
NRAS_p.Q61_62
PDS5B
PHF6
PPFIA2
PRPF8
PTEN
PTPN11
PTPRF
PTPRT
RAD21
RIT1
RUNX1
S100B
SETBP1
SF1
SF3B1
SMC1A
SMC3
SMG1
SPP1
SRSF2
STAG2
STAT5B
SUZ12
TET2
TP53
U2AF1
WT1
ZRSR2
add_8
add_11
add_13
add_21
add_22
del_20
del_3
del_5
del_7
del_9
del_12
del_13
del_16
del_17
del_18
minusy
t_v_11
t_10_21
t_12_13
t_12_17
t_12_22
t_13_19
t_15_16
t_15_17
t_16_17
t_16_21
t_17_19
t_17_21
t_1_12
t_1_14
t_1_16
t_1_17
t_1_19
t_1_3
t_1_4
t_1_5
t_1_6
t_2_17
t_2_3
t_2_5
t_2_7
t_2_9
t_3_16
t_3_21
t_3_5
t_3_7
t_3_9
t_4_12
t_4_21
t_4_9
t_5_12
t_5_17
t_5_9
t_6_9
t_7_16
t_7_17
t_7_8
t_8_10
t_8_13
t_8_16
t_8_17
t_8_21
t_9_11
t_9_13
t_9_17
t_9_22
complex
others_transloc
inv_3
inv_16

In [33]:
```
# png("figures/figures_miscellaneous/bdp1_comp.png",width=8000,height=8000,res=200)
dd_predicted_10_components <- get_prediction_result_dataframe(multi_output_cluster_10, df_hdp1)
tab <- get_table(dd_predicted_10_components[,'predicted_component'])
tab




# par(mfrow=c(12,1))
# plot_components_size(multi_output_cluster_10,8,4.5)
# plot_comp_distn(multi_output_cluster_10,
#                 cat_names  = colnames(df_hdp1),
#                 col        = "skyblue3",
#                 col_nonsig = "black",
#                 plot_title=c("Component 0 (going to HDP 2)","Component 1 (NPM1)","Component 2 (goin
g to HDP 2)","Component 3 (TP53-Complex)","Component 4 (going to HDP 2)",
#                             "Component 5 (t(8;21))","Component 6 (inv(16))","Component 7 (CEBPA_b
i)","Component 8 (t(15;17) & t(11))", "Component 9 (additions)","Component 10 (inv(3))")
#                                                 )
# dev.off()
# png("figures/figures_miscellaneous/bdp1_proba.png",width=3000,height=800,res=140)
plot_assignement_probability_by_component(dd_predicted_10_components)#+#+scale_fill_manual(values="bl
ue")
# ylab("Max Proba of assignment")+xlab("Predicted Component")   +
#     theme(plot.title=element_text(size=30,face="bold",hjust=0.5),axis.text=element_text(size=20),ax
is.title=element_text(size=25,face="bold")) +
#     ggtitle("Assignment probability of Top Component")
# dev.off()
```

```
Number of components: 10
Number of NA rows    : 51
```

A data.frame: 13 × 3

	values	count	freq
	<fct>	<int>	<chr>
2	1	833	38.7441860465116
3	2	418	19.4418604651163
4	3	229	10.6511627906977
5	4	128	5.95348837209302
6	5	114	5.30232558139535
7	6	114	5.30232558139535
1	0	96	4.46511627906977
9	8	61	2.83720930232558
12	NaN	51	2.37209302325581
8	7	50	2.32558139534884
11	10	29	1.34883720930233
10	9	27	1.25581395348837
13	-- total --	2150	100%



In [14]:
```
# NA Rows correspond to no defining events i.e all columns are 0 for those 51 patients
df_molecular <- read.table("data/aml_molecular_bdp.tsv")
initial_components <- cbind(df_molecular,add_first_second_predicted_component(multi_output_cluster_10
))
```

```
Number of components: 10
Number of NA rows    : 51
```

In [15]:
```
write.table(initial_components,file="data/initial_components_with_proba.tsv",sep='\t')
```

Rules : Patients that do not have molecular drivers defined by HDP will be going to HDP 2

Step 1: Defining feature in each component

In [16]:
```
tmp <- read.table("data/initial_components_with_proba.tsv")

tmp$initial_refined_component <- "not_assigned"

tmp[tmp$NPM1==1,]$initial_refined_component <- "NPM1"

tmp[tmp$initial_refined_component=="not_assigned" & (tmp$TP53==1 & tmp$complex==1),]$initial_refined_
component <- "TP53_complex"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==3 & (tmp$TP53==1
| tmp$complex==1),]$initial_refined_component <- "TP53_complex"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==5 & tmp$t_8_21==
1,]$initial_refined_component <- "t_8_21"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==6 & tmp$inv_16==
1,]$initial_refined_component <- "inv_16"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==7 & tmp$CEBPA_bi
==1,]$initial_refined_component <- "CEBPA_bi"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==8 & (tmp$t_v_11=
=1 | tmp$t_9_11==1),]$initial_refined_component <- "t_11"
tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==8 & tmp$t_15_17=
=1,]$initial_refined_component <- "t_15_17"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==9 & (tmp$add_8==
1 | tmp$add_11==1 | tmp$add_13==1 | tmp$add_21==1 | tmp$add_22==1),]$initial_refined_component <- "ad
ditions"

tmp[tmp$initial_refined_component=="not_assigned" & tmp$initial_predicted_component==10 & tmp$inv_3==
1,]$initial_refined_component <- "inv_3"

tmp[is.na(tmp$max_proba),]$initial_refined_component <- "no_events"
```

```
In [17]: t(data.frame(table(tmp$initial_refined_component)))
```

A matrix: 2 × 11 of type chr

Var1	additions	CEBPA_bi	inv_16	inv_3	no_events	not_assigned	NPM1	t_11	t_15_17	t_8_21	TP53_complex
Freq	21	35	85	13	51	915	682	31	19	99	199

Patients that did not pass the first test (not defining feature of their component)

```
In [18]: table_tmp <- t(data.frame(table(tmp[tmp$initial_refined_component=="not_assigned" & !is.element(tmp$i
nitial_predicted_component,c(0,2,4)),]$initial_predicted_component)))
rownames(table_tmp) <- c("Components","Number of Patients")
cat("Number of patients to test for second max proba in HDP 1: ")
cbind(table_tmp,c("Total",sum(table(tmp[tmp$initial_refined_component=="not_assigned" & !is.element(t
mp$initial_predicted_component,c(0,2,4)),]$initial_predicted_component))))
```

```
Number of patients to test for second max proba in HDP 1:
```

A matrix: 2 × 9 of type chr

Components	1	3	5	6	7	8	9	10	Total
Number of Patients	160	32	14	29	14	11	5	16	281

Step 2: Second Max Proba with defining features

In [19]:
```
### Get Min Proba for each component

min_proba_NPM1 = min(tmp[tmp$initial_predicted_component==1,]$max_proba,na.rm=T)

min_proba_TP53_complex = min(tmp[tmp$initial_predicted_component==3,]$max_proba,na.rm=T)

min_proba_t_8_21 = min(tmp[tmp$initial_predicted_component==5,]$max_proba,na.rm=T)

min_proba_inv_16 = min(tmp[tmp$initial_predicted_component==6,]$max_proba,na.rm=T)

min_proba_CEBPA_bi = min(tmp[tmp$initial_predicted_component==7,]$max_proba,na.rm=T)

min_proba_transloc = min(tmp[tmp$initial_predicted_component==8,]$max_proba,na.rm=T)

min_proba_additions = min(tmp[tmp$initial_predicted_component==9,]$max_proba,na.rm=T)

min_proba_inv_3 = min(tmp[tmp$initial_predicted_component==10,]$max_proba,na.rm=T)
```

In [20]:
```
## Refine patients with second max proba

threshold <- 0.75

tmp[tmp$initial_refined_component=="not_assigned"  ,]$initial_refined_component  <- apply(tmp[tmp$ini
tial_refined_component=="not_assigned" ,], 1,function(x) {
        if (all(is.na(x['second_predicted_component'])))
            return(NaN)
        else
            return(ifelse(x['second_predicted_component']==3 & x['second_max_proba']>=threshold*min_p
roba_TP53_complex & (x['complex']==1 | x["TP53"]==1),"TP53_complex",

                   ifelse(x['second_predicted_component']==5 & x['second_max_proba']>=threshold*min_p
roba_t_8_21 & x['t_8_21']==1 ,"t_8_21",

                   ifelse(x['second_predicted_component']==6 & x['second_max_proba']>=threshold*min_p
roba_inv_16 & x['inv_16']==1,"inv_16",

                   ifelse(x['second_predicted_component']==7  & x['second_max_proba']>=threshold*min_
proba_CEBPA_bi & x['CEBPA_bi']==1,"CEBPA_bi",

                   ifelse(x['second_predicted_component']==8 & x['second_max_proba']>=threshold*min_p
roba_transloc & (x['t_v_11']==1 | x['t_9_11']==1),"t_11",
                   ifelse(x['second_predicted_component']==8 & x['second_max_proba']>=threshold*min_p
roba_transloc & x['t_15_17']==1 ,"t_15_17",

                   ifelse(x['second_predicted_component']==9 & x['second_max_proba']>=threshold*min_p
roba_additions & (x['add_8']==1 | x['add_11']==1 | x['add_13']==1 | x['add_21']==1 | x['add_22']==1
),"additions",

                   ifelse(x['second_predicted_component']==10 & x['second_max_proba']>=threshold*min_
proba_inv_3 & x['inv_3']==1,"inv_3",
                   "not_assigned")))))))))
    })
t(data.frame(table(tmp$initial_refined_component)))
```

A matrix: 2 × 11 of type chr

Var1	additions	CEBPA_bi	inv_16	inv_3	no_events	not_assigned	NPM1	t_11	t_15_17	t_8_21	TP53_complex
Freq	24	39	88	13	51	888	682	41	20	99	205

In [21]:
```
write.table(tmp,"data/bdp1_assignment.tsv",sep='\t')
```

Patients in category not assigned do not have defining features of their component nor of their second predicted component. They will also go in BDP 2.

II- BDP2 : BDP reapplied on 888 Patients

888 Patients from Component 0,2 and 4 + Patients from other components that do not have defining features from their component.

In [22]:
```
hdp1_assignment <- read.table('data/bdp1_assignment.tsv')

# We reapply HDP to those patients :

df_to_recluster <- hdp1_assignment[hdp1_assignment$initial_refined_component=="not_assigned",c(genes_
columns,cytos_columns)]   ## Reapply HDP only on not assigned patients and keep only gene and cyto co
lumns
```

In [23]:
```
num_cols = ncol(df_to_recluster[colSums(df_to_recluster) > 0])  ## keep only column that have at leas
t a mutation (otherwise HDP is not applicable)
num_cols
bin <- function(x){
    set.seed(123)
  (rbinom(1, num_cols, mean(x))+1)/num_cols
}

###Normal

normal <- function(x){
    set.seed(123)
  abs(rnorm(1,mean(x),sd(x)))
}

###Poisson

poisson <- function(x){
    set.seed(123)
  (rpois(num_cols,1)+1)/num_cols
}

###Uniform equally over all columns

equally <- function(x){
    set.seed(123)
  1/num_cols
}

###Repet 1

repet <- function(x){
    set.seed(123)
  1
}

binomial <- unlist(sapply(df_to_recluster[colSums(df_to_recluster) > 0],bin))
gaussian <- unlist(sapply(df_to_recluster[colSums(df_to_recluster) > 0],normal))
pois <- as.numeric(unlist(sapply(df_to_recluster[colSums(df_to_recluster) > 0],poisson)))
unif <- unlist(sapply(df_to_recluster[colSums(df_to_recluster) > 0],equally))
```

```
129
```

In [24]:
```
# HDP2 Parameters

data <- df_to_recluster[colSums(df_to_recluster) > 0]
posterior_samples <- 150
initial_clusters <- 5
burn <- 5000
chains <- 3
base_dist <- prepare_distributions(df_to_recluster[colSums(df_to_recluster) > 0])$gaussian
aa <- 2
ab <-6
```

In [25]:
```
multi_output <- launch(df_to_recluster[colSums(df_to_recluster) > 0],base_dist,initial_clusters,burn,
posterior_samples,chains,aa,ab)

multi_output_recluster_5 <- extract_components(multi_output)
```

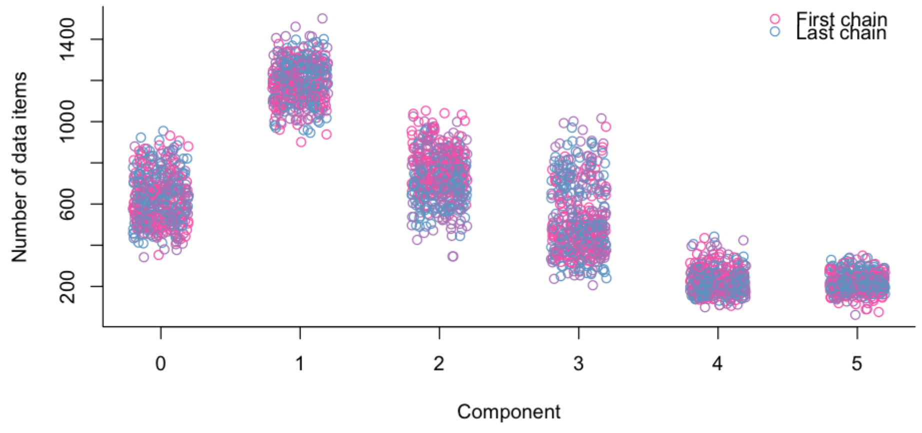
```
Initialise HDP on a 888 x 129 dataframe
  → create HDP structure... done!
  → add DP node for each patient... done!
  → assign the data to the nodes... done!
### Experiment 1 (seed = 100) ###
Activate HDP nodes and run posterior sampling
  → activate HDP nodes... done!
  → run posterior sampling...
[1] "5000 burn-in iterations in 0.2 mins"

### Experiment 2 (seed = 200) ###
Activate HDP nodes and run posterior sampling
  → activate HDP nodes... done!
  → run posterior sampling...
[1] "5000 burn-in iterations in 0.2 mins"

### Experiment 3 (seed = 300) ###
Activate HDP nodes and run posterior sampling
  → activate HDP nodes... done!
  → run posterior sampling...
[1] "5000 burn-in iterations in 0.2 mins"

Object of class hdpSampleMulti
 Number of chains: 3
 Total posterior samples: 450
 Components: NO. Run hdp_extract_components
 ----------
 Final hdpState from first chain:
Object of class hdpState
 Number of DP nodes: 889
 Index of parent DP: 0 1 1 1 1 1 1 1 1 1 ...
 Number of data items per DP: 0 8 6 5 4 3 4 4 7 4 ...
 Index of conparam per DP: 1 1 1 1 1 1 1 1 1 1 ...
 Conparam hyperparameters and current value:
           Shape Rate   Value
Conparam 1     2    6 2.65891
 Number of data categories: 129
 Number of clusters: 34
 Initialised with 5 clusters, using random seed 100
Extract HDP components from posterior sampling
  → extract components... done!
* 5 components found
```

In [26]:
```
dd_predicted_5_components <- get_prediction_result_dataframe(multi_output_recluster_5, df_final)

tab <- get_table(dd_predicted_5_components[,'predicted_component'])

tab

plot_components_size(multi_output_recluster_5,8,4.5)

set_notebook_plot_size(16,2.6)

plot_comp_distn(multi_output_recluster_5,
                    cat_names   = colnames(df_to_recluster[colSums(df_to_recluster) > 0]),
                    col         = "skyblue3",
                    col_nonsig = "black",
                    plot_title=c("Component 0 (mix)","Component 1 (chr splicing)","Component 2 (chr s
plicing)","Component 3 (DNMT3A IDH1-2)","Component 4 (chr splicing)","Component 5 (WT1 and t(6;9))"))
```

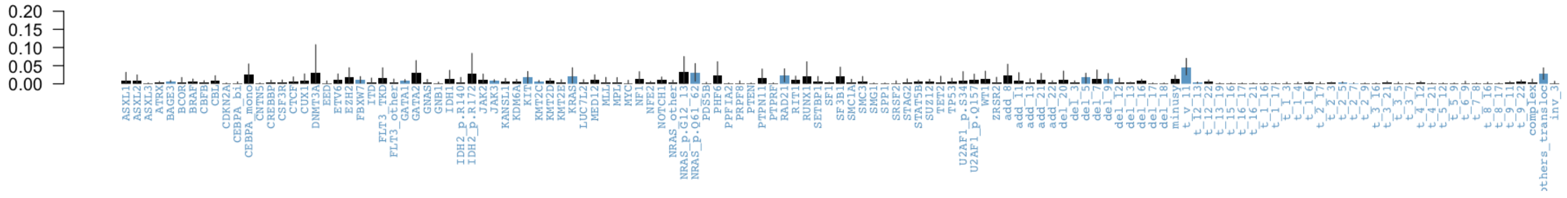
```
Number of components: 5
Number of NA rows    : 0
```

A data.frame: 7 × 3

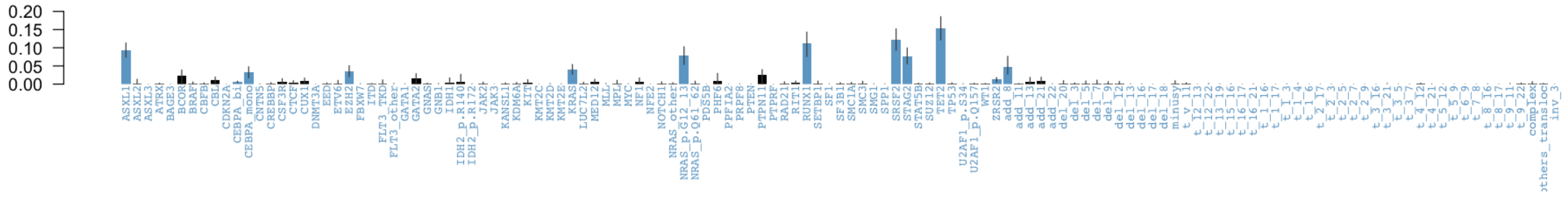
	values	count	freq
	<fct>	<int>	<chr>
2	1	319	35.9234234234234
3	2	228	25.6756756756757
1	0	144	16.2162162162162
4	3	101	11.3738738738739
6	5	62	6.98198198198198
5	4	34	3.82882882882883
11	-- total --	888	100%



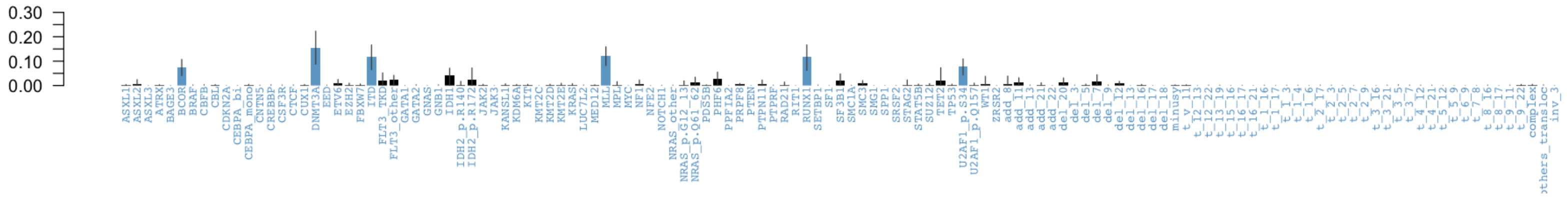
Component 0 (mix)



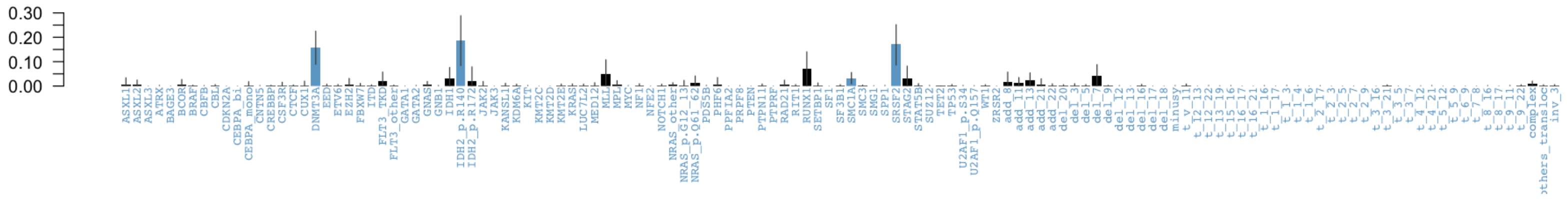
Component 1 (chr splicing)



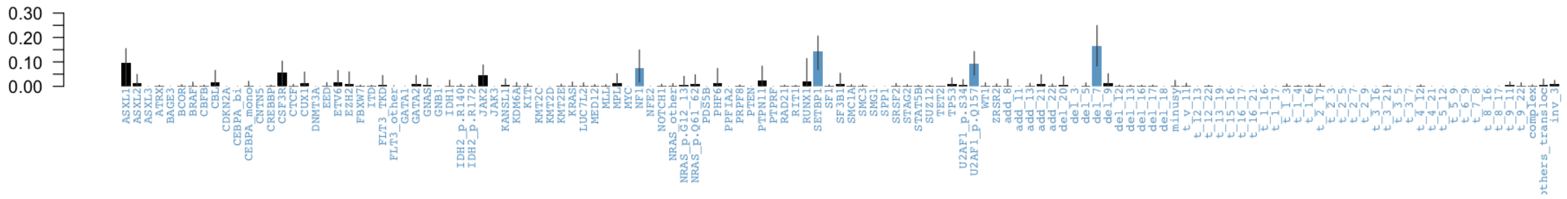
Component 2 (chr splicing)



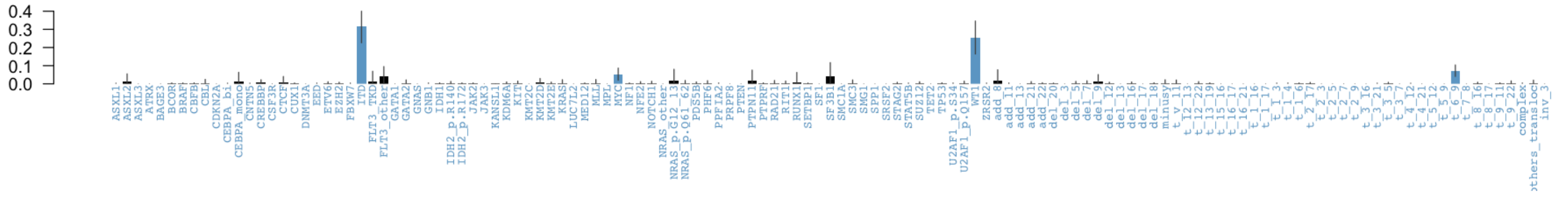
Component 3 (DNMT3A IDH1-2)



Component 4 (chr splicing)



Component 5 (WT1 and t(6;9))



In [27]:
```
# png("figures/figures_miscellaneous/bdp2_comp.png",width=8000,height=8000,res=200)
dd_predicted_5_components <- get_prediction_result_dataframe(multi_output_recluster_5, df_final)

tab <- get_table(dd_predicted_5_components[,'predicted_component'])

tab



# par(mfrow=c(12,1))
# plot_components_size(multi_output_recluster_5,8,4.5)
# plot_comp_distn(multi_output_recluster_5,
#                      cat_names  = colnames(df_to_recluster[colSums(df_to_recluster) > 0]),
#                      col        = "skyblue3",
#                      col_nonsig = "black",
#                      plot_title=c("Component 0 (mix)","Component 1 (chr splicing)","Component 2 (chr
splicing)","Component 3 (DNMT3A IDH1-2)","Component 4 (chr splicing)","Component 5 (WT1 and t(6;
9))"))

# dev.off()
# png("figures/figures_miscellaneous/bdp2_proba.png",width=3000,height=800,res=140)
# plot_assignement_probability_by_component(dd_predicted_5_components)+#+scale_fill_manual(values="bl
ue")
# ylab("Max Proba of assignment")+xlab("Predicted Component")   +
#     theme(plot.title=element_text(size=30,face="bold",hjust=0.5),axis.text=element_text(size=20),ax
is.title=element_text(size=25,face="bold")) +
#     ggtitle("Assignment probability of Top Component")
# dev.off()
```

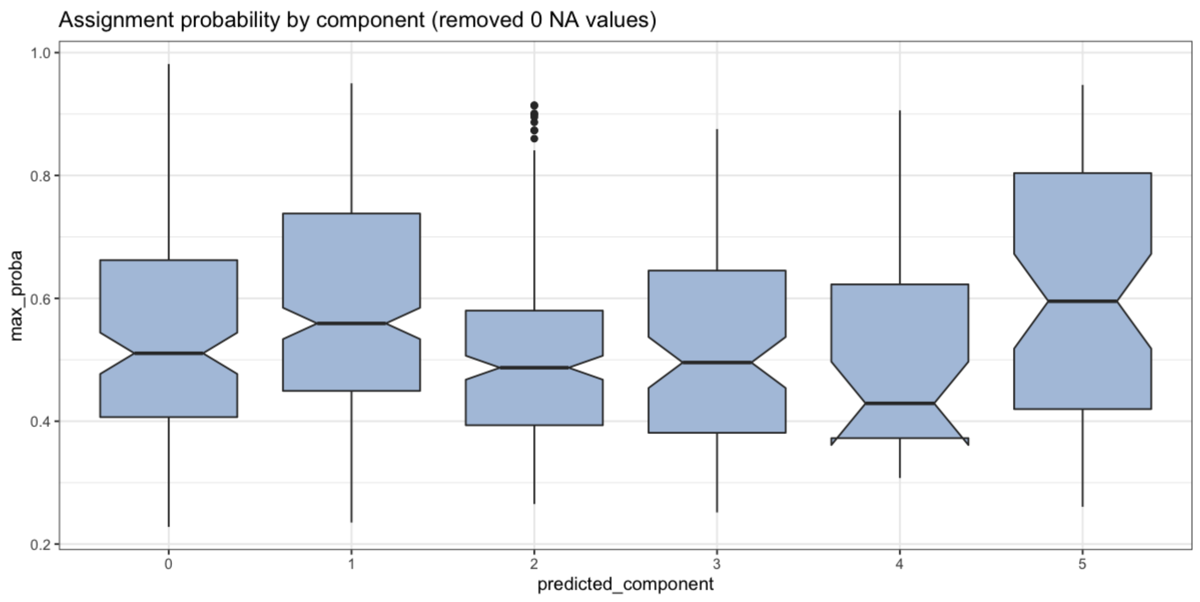
```
Number of components: 5
Number of NA rows   : 0
```

A data.frame: 7 × 3

	values	count	freq
	<fct>	<int>	<chr>
2	1	319	35.9234234234234
3	2	228	25.6756756756757
1	0	144	16.2162162162162
4	3	101	11.3738738738739
6	5	62	6.98198198198198
5	4	34	3.82882882882883
11	-- total --	888	100%

```
In [28]: plot_assignement_probability_by_component(dd_predicted_5_components)
```

```
notch went outside hinges. Try setting notch=FALSE.
```


Assignment probability by component (removed 0 NA values)
max_proba
predicted_component