Analysis

January 10, 2019

```
In [81]: library(Homo.sapiens)
         library(taRifx) ## Removes factors
         library(scales)
         library(SchramekLOH)
         library(gplots)
         library(IdeoViz)
         library(reshape)
In [133]: #detach("package:SchramekLOH", unload=TRUE)
          #library(SchramekLOH)
Attaching package: 'SchramekLOH'
The following objects are masked _by_ '.GlobalEnv':
    df.ex, parseIdsByMutation, seg, snp6, visOneGene
The following object is masked from 'package:OrganismDbi':
    mapIds
The following object is masked from 'package: Annotation Dbi':
   mapIds
```

1 Setup

Loading in all the precomputed data files

```
data("snp6") # snp6
data("Affyseg") # affyseg
data("TCGAseg") # seg
```

Setting up some of the paths

Determine whether to use the TCGA Segs or the Affymetrix SNP6 Segs (same that the bird-seeds originate from)

```
In [85]: #### Read in Birdseed + Segs ####
     use.affy <- FALSE
     if(use.affy) seg <- affyseg</pre>
```

In [86]: head(seg)

ID	chrom	loc.start	loc.end	num.mark	seg.mean
TCGA-CN-6010-01	1	3218610	70988682	38435	-0.0543
TCGA-CN-6010-01	1	70990192	71001138	11	0.1953
TCGA-CN-6010-01	1	71002192	104005432	19689	-0.2485
TCGA-CN-6010-01	1	104009909	104613056	160	-0.0488
TCGA-CN-6010-01	1	104613622	149881398	9504	-0.2554
TCGA-CN-6010-01	1	149882014	247813706	61340	0.1959

2 Preprocess

2.1 Ordering all the data structures

```
In [87]: #### Chromosome order datasets ####
    seg$chrom <- gsub("(chr).*\\1", "\\1", paste0("chr", seg$chrom))
    seg.ids <- split(seg, f=seg$ID)
    seg.chr <- lapply(seg.ids, function(seg.i){
        seg.tmp <- split(seg.i, f=seg.i$chrom)
        chrom.ord <- match(paste0("chr", c(1:22)), names(seg.tmp))
        seg.tmp[chrom.ord]
    })</pre>
```

```
In [97]: head(seg.chr[['BALMS_p_TCGAb54and67_SNP_N_GenomeWideSNP_6_A03_730402']][[
          head(seg.chr[['TCGA-4P-AA8J-01']][['chr3']])
```

NULL

```
ID
                        chrom loc.start
                                         loc.end
                                                   num.mark
                                                             seg.mean
      TCGA-4P-AA8J-01 chr3
                                2212571
                                         63587411
                                                   35293
                                                              -0.2729
60692
60693
     TCGA-4P-AA8J-01
                        chr3
                                63588304 63597382
                                                              -1.8286
60694 | TCGA-4P-AA8J-01 chr3
                                63597937 74146967
                                                   7604
                                                              -0.2720
60695 | TCGA-4P-AA8J-01 chr3
                                74148159 74151871
                                                              -1.7897
                                                   3
60696
      TCGA-4P-AA8J-01 chr3
                                74155664 90485962 7974
                                                              -0.2668
60697 | TCGA-4P-AA8J-01 chr3
                                93734671 97956918 2134
                                                              0.0384
```

```
In [89]: #### Map Probesets to Genomic Loci ####
    if(exists("ref.probe.ord")) {
        snp6 <- snp6[match(ref.probe.ord, snp6$V4),]
        snp6.ord <- snp6[,c(4, 1:3)]
        colnames(snp6.ord) <- c("probeset_id", "chrom", "start", "end")

        snp6.chr <- split(snp6.ord, f=snp6.ord$chrom)
        bs.chr <- split(as.data.frame(df.bs), snp6.ord$chrom)
        goi.df <- getGeneLoci(goi)
        goi.chr <- split(goi.df, f=goi.df$chr)

        chrom.ord <- match(paste0("chr", c(1:22, "X", "Y")), names(snp6.chr))
        snp6.chr <- snp6.chr[chrom.ord]
        bs.chr <- bs.chr[chrom.ord]
}</pre>
```

2.2 Formatting the Gene expression data

```
In [90]: #### Expression analysis
if(exists("df.ex")) {
    ## Generate z-score per gene
    z <- function(x) { (x - mean(x, na.rm=TRUE)) / sd(x, na.rm=TRUE) }
    z.ex <- data.frame(t(apply(df.ex, 1, z)), stringsAsFactors=FALSE)

## Map Genes to Genomic Loci ##
    ord <- match(rownames(df.ex), gaf$V2)
    gaf.ord <- gaf[ord, c("V2", "V17")]
    gaf.ord$chr <- gsub(":.*", "", gaf.ord$V17)
    gaf.ord$start <- as.numeric(gsub("^.*:", "", gsub("-.*", "", gaf.ord$V]

## Reorder all the matrices into genomic loci numerical order
    chr.ord <- paste0("chr", c(1:22, "X", "Y"))
    gaf.ord <- gaf.ord[order(gaf.ord$start),]
    gaf.ord <- gaf.ord[order(match(gaf.ord$chr, chr.ord)), ]</pre>
```

```
ord <- match(gaf.ord$V2, rownames(df.ex))
df.ex <- df.ex[ord,]
z.ex <- z.ex[ord,]

## Order the list by chromosomes
gaf.chr <- split(gaf.ord, f=gaf.ord$chr)
z.chr <- split(z.ex, f=gaf.ord$chr)

chrom.ord <- match(paste0("chr", c(1:22, "X", "Y")), names(gaf.chr))
gaf.chr <- gaf.chr[chrom.ord]
z.chr <- z.chr[chrom.ord]
}</pre>
```

2.3 Comparison of overlapping Expression and Seg arrays

3 Analysis

504

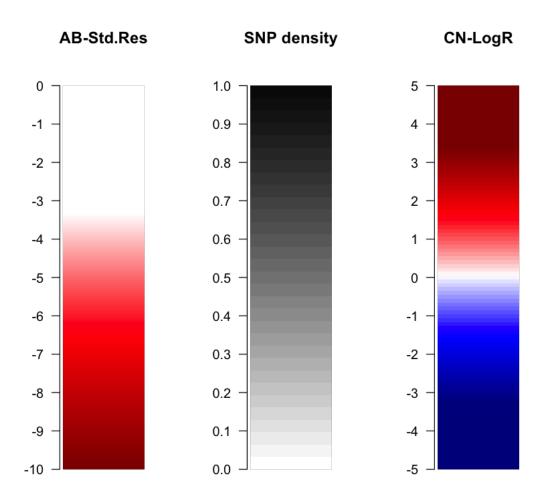
3.1 Visualization and generate StdRes

Setting up the colours that will be used for all visualizations

Visualization for the colour bars and ranges used

```
In [94]: #pdf(file.path(plotsdir, "legend.pdf"), width=6)
    null <- split.screen(c(1,3))
    screen(1); color.bar(p, min=0, max=-10, title="AB-Std.Res")</pre>
```

```
screen(2); color.bar(r, min=0, max=1, title="SNP density")
screen(3); color.bar(cn, min=-5, max=5, title="CN-LogR")
close.screen(all.screens=TRUE)
#dev.off()
```



Summarize the standardized residuals data into data frames

```
In [744]: all.stdres <- lapply(sample.stdres, function(i) i[['all']])</pre>
           names(all.stdres) <- colnames(bs.chr[[1]])</pre>
           ## Reduce the gene to a single segment
           sample.stdres.bkup <- sample.stdres</pre>
           sample.stdres <- lapply(sample.stdres, function(i) {</pre>
             single.j <- sapply(split(i[['genes']], f=i[['genes']]$gene), function('</pre>
               uniq.j <- apply(j, 2, unique)</pre>
               if(any(sapply(uniq.j[c('seg.start', 'seg.end', 'seg.mean')], length)
                 uniq.j[['seg.start']] <- min(uniq.j[['seg.start']])</pre>
                 uniq.j[['seg.end']] <- max(uniq.j[['seg.end']])</pre>
                 uniq.j[['seg.mean']] <- mean(uniq.j[['seg.mean']])</pre>
               }
               sapply(uniq.j, function(x) x)
             remove.factors(data.frame(t(single.j)))
           })
           names (sample.stdres) <- colnames (bs.chr[[1]])</pre>
In [100]: head(all.stdres[['FISTS_p_TCGA_b107_121_SNP_N_GenomeWideSNP_6_D08_777884
                                         stdres
                 chrom start
                                end
                                1000797 -2.5980762
       [0,1e+06]
                chr1
   (1e+06,2e+06]
                chr1
                        1000797
                                2001595
                                        -0.9878292
   (2e+06,3e+06] | chr1
                        2001595 3002393 -0.3333333
In [99]: head(sample.stdres[['FISTS_p_TCGA_b107_121_SNP_N_GenomeWideSNP_6_D08_77788
             gene
                       chr
                              gene.start gene.end bin.start bin.end
                                                                    seg.start
                                                                              seg.end
                                                                                       seg.n
                                        59042177 58814431 59621572 54814864
                                                                              74252408
                                                                                       0.086
   ADAM10 | ADAM10
                       chr15 58888510
             AIUBA
                                        23451848 22652964 23466738
                                                                    20501368
                                                                                       -0.26
     AIUBA
                       chr14 23440410
                                                                              35724239
                                                                                       -0.28
       JAG1 | JAG1
                       chr20 10618332
                                        10654694 10038090 11035720 455764
                                                                              25990441
```

3.2 Generate 'Attributes' files for use in IGV

Summarize all the Standardized Residuals into LOH/Het value annotations for IGV visualization

load(file.path(tmpdir, paste0("tmp", use.affy, ".RData")))

In [98]: #save(all.stdres, sample.stdres, sample.stdres.bkup, file=file.path(tmpdi

	TRACK_ID	ADAM10	AJUBA	JAG1	JAG2	NOTCH1	NOTCH2	NOTCH3	N
loh.val	TCGA-CN-6011-01	Het	Het	Het	LOH	Het	LOH	Het	H
loh.val1	TCGA-CN-6012-01	Het	LOH	LOH	LOH	LOH	LOH	Het	H
loh.val2	TCGA-CN-6016-01	Het	Het	LOH	LOH	LOH	Het	LOH	L
loh.val3	TCGA-CN-6018-01	Het	Het	LOH	LOH	LOH	Het	Het	L
loh.val4	TCGA-CN-6019-01	LOH	LOH	LOH	LOH	Het	Het	LOH	L
Write and save the data structures									

3.3 Generate contigency tables and test for significance

Initialize the contigency table to be used for quick reference later

Available mutations to compare for LOH, where "ADAM10" actually means "ADAM10_LOH"

```
In [105]: print(names(ctbl))
```

```
[1] "NOTCH1_CNA"
                      "NOTCH1_MUT"
                                       "NOTCH1_FUSION" "NOTCH2_CNA"
[5] "NOTCH2_MUT"
                      "NOTCH2_FUSION"
                                       "NOTCH3_CNA"
                                                        "NOTCH3_MUT"
[9] "NOTCH3_FUSION"
                      "NOTCH4_CNA"
                                       "NOTCH4_MUT"
                                                        "NOTCH4_FUSION"
[13] "JAG1_CNA"
                      "JAG1_MUT"
                                       "JAG1_FUSION"
                                                        "JAG2_CNA"
[17] "JAG2_MUT"
                                       "DLL1_CNA"
                      "JAG2_FUSION"
                                                        "DLL1_MUT"
[21] "DLL1_FUSION"
                      "ADAM10_CNA"
                                       "ADAM10_MUT"
                                                        "ADAM10_FUSION"
[25] "AJUBA_CNA"
                      "AJUBA_MUT"
                                       "AJUBA_FUSION"
                                                        "ADAM10"
[29] "AJUBA"
                                       "JAG2"
                      "JAG1"
                                                        "NOTCH1"
[33] "NOTCH2"
                      "NOTCH3"
                                       "NOTCH4"
```

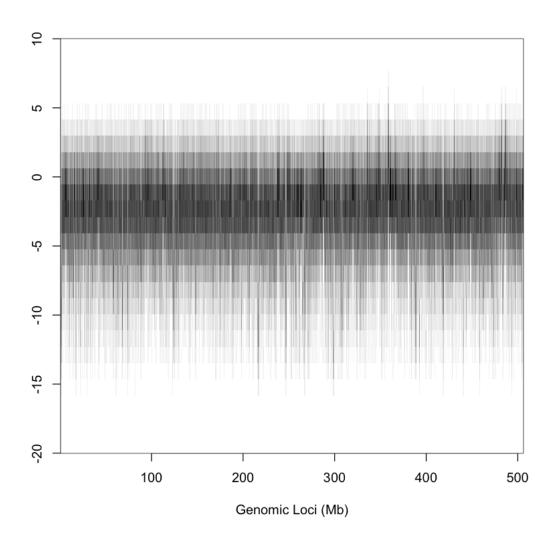
Run the chi-squared analysis on the samples that are of interest

```
$Contigency
j
i HETLOSS HOMDEL no_alteration
Het 53 0 183
LOH 58 1 211
$p
[1] 0.8926257
$Std.Res
i HETLOSS no_alteration
 Het 0.2426701 -0.2426701
 LOH -0.2426701 0.2426701
$Contigency
  j
i HOMDEL no_alteration
Het 4 245
LOH 7 250
$р
[1] 0.5777054
$Std.Res
i HOMDEL no_alteration
Het -0.8616207 0.8616207
LOH 0.8616207 -0.8616207
$Contigency
i HETLOSS HOMDEL no_alteration
Het 24 1 126
LOH 51 0 304
[1] 0.7377338
$Std.Res
i HETLOSS no_alteration
 Het 0.4717808 -0.4717808
```

LOH -0.4717808

0.4717808

3.4 StdRes plots



3.5 Ideogram of HetLoss over Gene of Interest

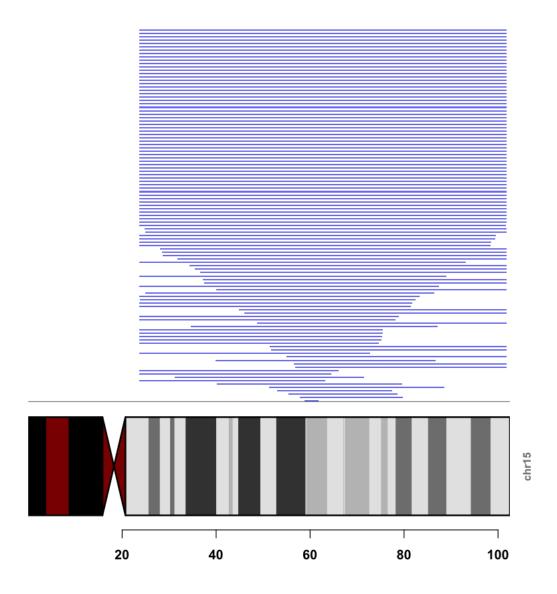
Set up the Ideogram hg19 reference

```
In [107]: ideo_hg19 <- getIdeo("hg19")</pre>
```

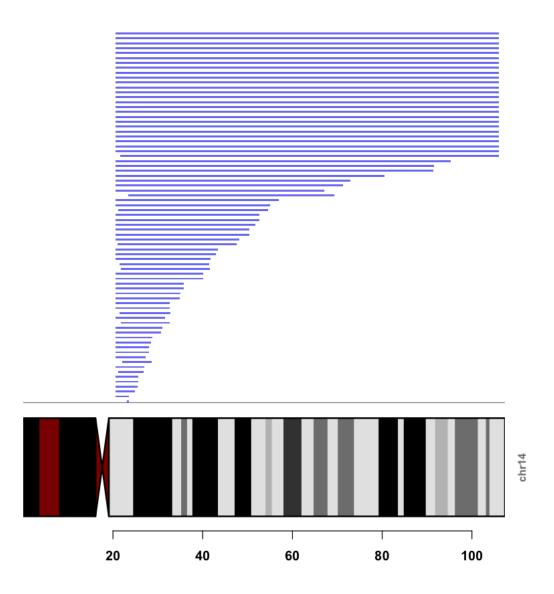
Visualize each gene of interest in their ideogram

```
In [30]: for(gene in rownames(sample.stdres[[1]])){
      pdf(file.path(outdir, paste0("ideo_", gene, ".pdf")), height=20)
      plotIdeoGene(ideo_hg19, sample.stdres, gene, thresh=-0.1)
      dev.off()
}
```

In [28]: plotIdeoGene(ideo_hg19, sample.stdres, 'ADAM10', thresh=-0.1)



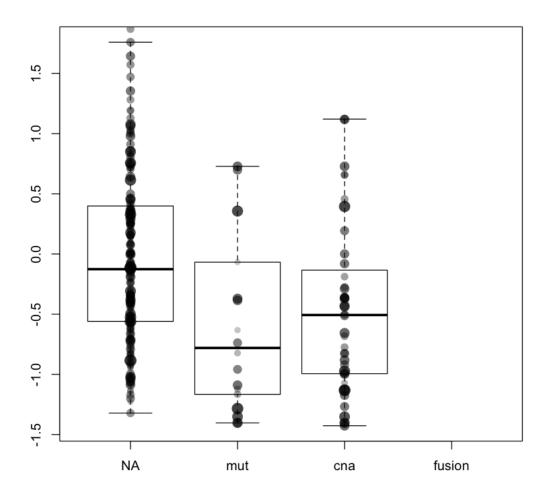
In [29]: plotIdeoGene(ideo_hg19, sample.stdres, 'AJUBA', thresh=-0.1)



3.6 Expression of AJUBA Mutants

Grab gene expression for all samples and assign them to their proper data structures. Then, separate the samples based on their mutations

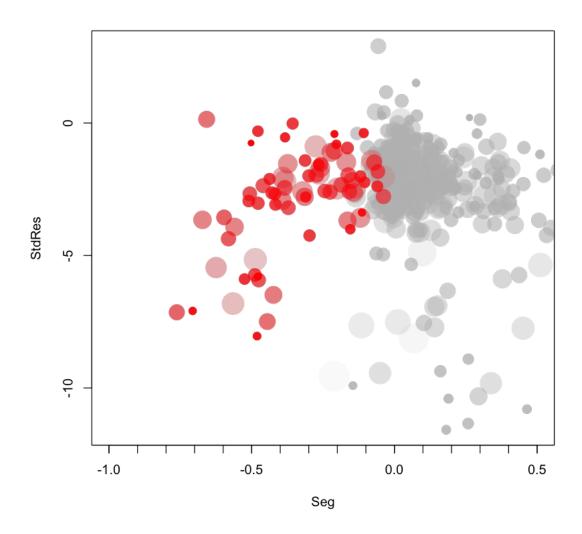
```
x <- sapply(seq_along(ex.by.mut), function(x){</pre>
                   ex <- ex.by.mut[[x]]</pre>
                   points (x=rep(x, nrow(ex)), y=ex[, ex.id],
                          pch=16, col=alpha("black", rescale(as.numeric(ex[,4]), to=
                          cex=rescale(as.numeric(ex[,4]), to=c(1,2)))
               })
               NULL
           }
In [110]: ex.by.mut <- parseIdsByMutation('AJUBA', getGeneExp('JUB'),</pre>
                                            seg.ids=seg.ids, lo.q=0.1, hi.q=0.9)
          null <- .mutBoxplot(ex.by.mut)</pre>
          lapply(ex.by.mut, head, 3)
Warning message in min(x):
"no non-missing arguments to min; returning Inf"Warning message in max(x):
"no non-missing arguments to max; returning -Inf''Warning message in min(x):
"no non-missing arguments to min; returning Inf''Warning message in max(x):
"no non-missing arguments to max; returning -Inf"
                                    JUB.84962 IQR.90.
            TRACK_ID | Alt
      TCGA-BA-4074-01
                       no alteration -1.0212289 0.4705
$'NA'
      TCGA-BA-4076-01
                       no_alteration
                                    -0.1099163
                                              1.2144
      TCGA-BA-4077-01 | no_alteration -0.3082486 1.0026
             TRACK_ID | AJUBA_MUT
                                       JUB.84962
                                                  IQR.90.
       TCGA-BA-5556-01
                        R428Q I304Dfs*2
                                       -0.6325205
                                                  0.1064
$mut
      TCGA-BB-A5HY-01
                        S230Ffs*76
                                       NA
                                                  0.68
      TCGA-BB-A6UO-01 | V264Lfs*2
                                       NA
                                                  0.71
           TRACK ID | AJUBA CNA
                                  JUB.84962
                                             IQR.90.
     TCGA-4P-AA8J-01
                      HETLOSS
                                   NA
                                              0.2877
$cna
     TCGA-BA-4075-01
                      HETLOSS
                                   -0.2885436
                                              0.6765
     TCGA-BA-4078-01
                     HETLOSS
                                    -0.6562828
                                             0.7544
$fusion TRACK_ID | AJUBA_FUSION JUB.84962
                                             IQR.90.
```



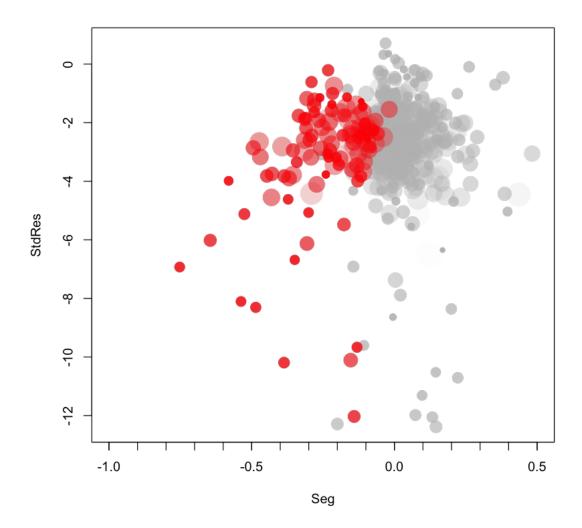
	TRACK_ID	AJUBA_MUT	JUB.84962	IQR.90.
13	TCGA-CV-6003-01	T337_C341del	2.65706121	0.7254
11	TCGA-CR-6493-01	C406S	0.72805934	0.6963
25	TCGA-CX-7082-01	H360Y	0.69803062	0.6209
6	TCGA-CN-6997-01	R50Efs*192	0.35677236	0.9341
15	TCGA-CV-7099-01	H423Y	-0.06802904	0.068
10	TCGA-CR-6491-01	Q103*	-0.37074236	0.7302
18	TCGA-CV-7424-01	D108Rfs*16	-0.38532999	0.6718
1	TCGA-BA-5556-01	R428Q I304Dfs*2	-0.63252046	0.1064
14	TCGA-CV-6950-01	Q353*	-0.73796721	0.5662
4	TCGA-CN-4738-01	A351Qfs*39	-0.82308846	0.1903
30	TCGA-HD-7753-01	R324Gfs*84	-0.95843514	0.5462
29	TCGA-DQ-7588-01	R428*	-1.08991576	0.6774
7	TCGA-CN-6998-01	N433I	-1.12249891	0.2421
5	TCGA-CN-6018-01	C270Wfs*10	-1.16581195	0.2961
17	TCGA-CV-7418-01	E279*	-1.28425163	1.0081
12	TCGA-CV-5435-01	L280Afs*26	-1.35174099	0.8639
16	TCGA-CV-7177-01	E305Sfs*105	-1.40261590	0.8049
19	TCGA-CV-7432-01	R293*	-1.40352314	0.5513
2	TCGA-BB-A5HY-01	S230Ffs*76	NA	0.68
3	TCGA-BB-A6UO-01	V264Lfs*2	NA	0.71
8	TCGA-CN-A63V-01	C426Y	NA	0.2171
9	TCGA-CQ-5327-01	Q76*	NA	0.0558
20	TCGA-CV-A45Q-01	Q103* S302Wfs*108	NA	0.2981
21	TCGA-CV-A460-01	R293Lfs*13	NA	0.3748
22	TCGA-CV-A463-01	R371*	NA	0.3251
23	TCGA-CV-A6JM-01	E253Gfs*53	NA	0.6672
24	TCGA-CV-A6K2-01	G370R T361Sfs*48	NA	0.0437
26	TCGA-D6-A6EN-01	I339Nfs*50	NA	0.043
27	TCGA-D6-A6EP-01	X414_splice	NA	1.0883
28	TCGA-D6-A74Q-01	Q362*	NA	0.6643
31	TCGA-P3-A5QF-01	E507Kfs*54	NA	0.8036
32	TCGA-UF-A71D-01	X414_splice	NA	0.6532
33	TCGA-UF-A7JF-01	R77Pfs*164	NA	0.6197

3.7 Association between LRR and StdRes of HETLOSS Regions

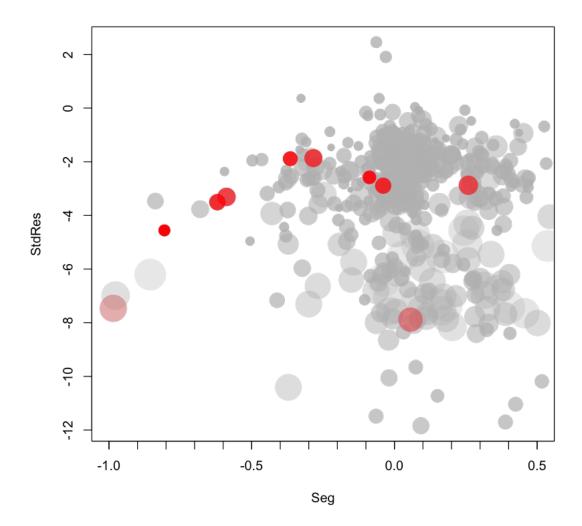
```
In [112]: gene <- 'AJUBA'
    mut <- paste0(gene, "_CNA")
    rge <- 5
    visOneGene(gene, rge, mut)</pre>
```



```
In [73]: gene <- 'ADAM10'
    mut <- paste0(gene, "_CNA")
    rge <- 5
    visOneGene(gene, rge, mut)</pre>
```



```
In [79]: gene <- 'NOTCH1'
    mut <- paste0(gene, "_CNA")
    rge <- 5
    visOneGene(gene, rge, mut)</pre>
```



For interest, there was one AJUBA case that had Homozygous Deletion, and one AJUBA case where the logRRatio was EXTREMELY low. I wanted to see the details of these cases

```
3.7.1 ——
```

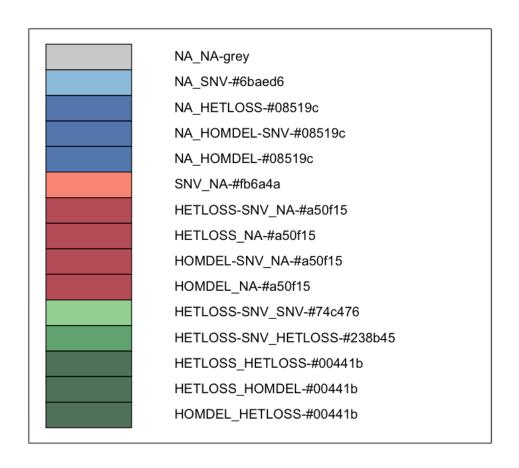
Sample with a -3.0 segment

	TRACK_ID	NOTCH1_CNA	NOTCH1_MUT	NOTCH1_FU	JSION	NOTCH2_	CNA NO
323	TCGA-CN-6988-01	no_alteration	no_alteration	no_alteration	l	no_alterati	on no
				mean	sd	seg	HETLOS
MIRES_p_TCGA_151_SNP_N_GenomeWideSNP_6_G03_831548				8 -10.39918	2.15365	8 -2.944	FALSE

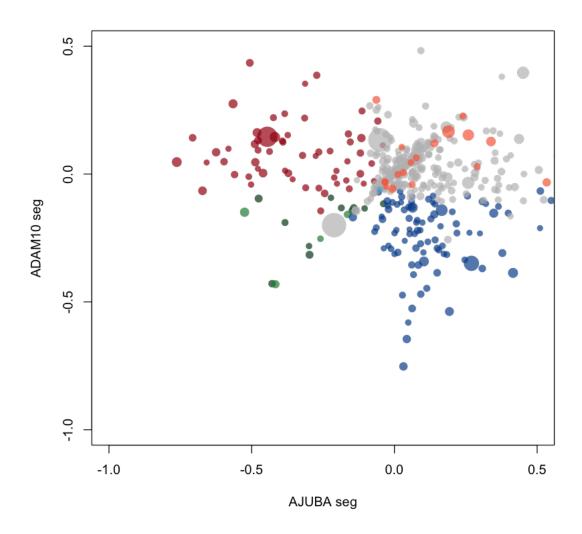
3.8 Comparison of Seg/LOH between two genes

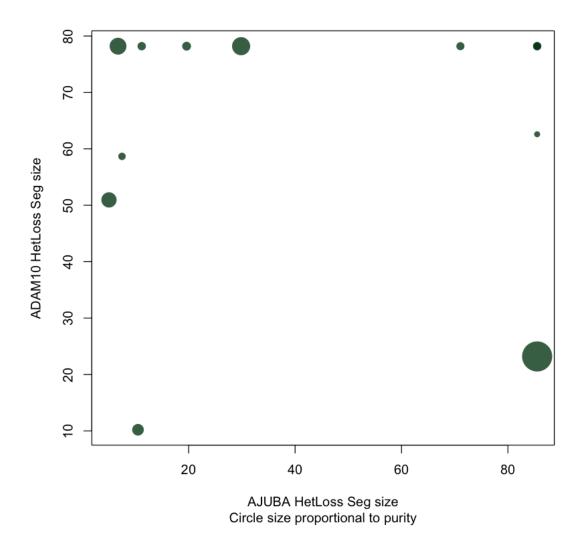
print("Low LRR case")

Create a colour schema for the unique ids (UID)

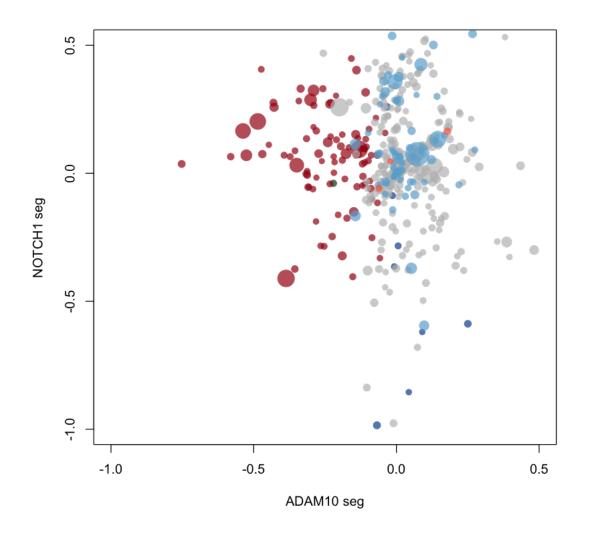


UID	TCGA_ID	TRACK_ID
HETLOSS_HETLOSS	TCGA-CQ-5332-01	FISTS_p_TCGA_b107_121_SNP_N_GenomeWideSNP_6_B02
HETLOSS_HETLOSS	TCGA-CV-5966-01	FISTS_p_TCGA_b107_121_SNP_N_GenomeWideSNP_6_A11
HETLOSS_HETLOSS	TCGA-H7-A76A-01	UNDID_p_TCGA_353_354_355_37_NSP_GenomeWideSNP_

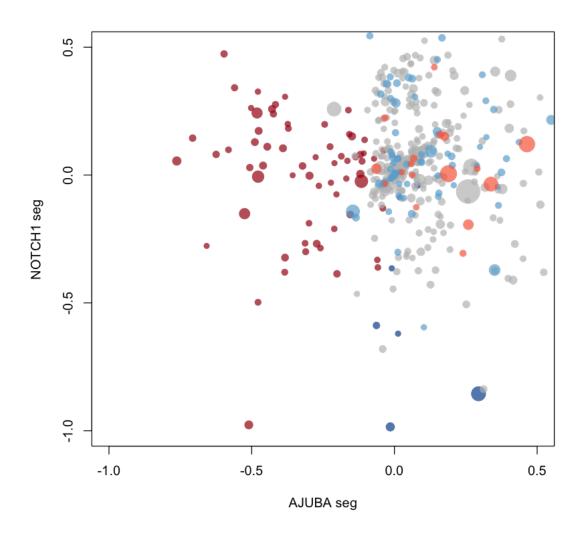




	UID	TCGA_ID	TRACK_ID
2	HETLOSS_HOMDEL-SNV	TCGA-QK-A8ZA-01	MESNE_p_TCGAb_401_02_03_04_05_N_Genom
10	HETLOSS_NA	TCGA-CV-6441-01	FISTS_p_TCGA_b107_121_SNP_N_GenomeWid
28	HETLOSS_NA	TCGA-KU-A66T-01	RICES_p_TCGA_Batch_310_311_NSP_GenomeV
33	HETLOSS_NA	TCGA-CV-7104-01	PEAKY_p_TCGA_b164_SNP_N_GenomeWideSI
50	HETLOSS_NA	TCGA-P3-A6T3-01	UNDID_p_TCGA_353_354_355_37_NSP_Genom
60	HETLOSS_NA	TCGA-CV-7435-01	MAULS_p_TCGA_189_190_SNP_N_GenomeWid
85	HETLOSS_NA	TCGA-UF-A719-01	CUTCH_p_TCGAb_355_37_52_NSP_GenomeWi
105	HETLOSS_SNV	TCGA-DQ-5631-01	GROVE_p_TCGA_b145_153_SNP_N_GenomeW
114	NA_HOMDEL	TCGA-BB-4224-01	BALMS_p_TCGAb54and67_SNP_N_GenomeWi
115	NA_HOMDEL	TCGA-P3-A5QA-01	LEGIT_p_TCGA_300_301_302_N_GenomeWides



	UID	TCGA_ID	TRACK_ID
3	HETLOSS_NA	TCGA-CR-5247-01	PEAKY_p_TCGA_b164_SNP_N_GenomeWideSNP_6_B09_863
4	HETLOSS_NA	TCGA-F7-7848-01	MAULS_p_TCGA_189_190_SNP_N_GenomeWideSNP_6_G10
7	HETLOSS_NA	TCGA-CN-4735-01	BALMS_p_TCGAb54and67_SNP_N_GenomeWideSNP_6_A03
17	HETLOSS_NA	TCGA-CV-7435-01	MAULS_p_TCGA_189_190_SNP_N_GenomeWideSNP_6_G12
18	HETLOSS_NA	TCGA-CN-A63U-01	RICES_p_TCGA_Batch_310_311_NSP_GenomeWideSNP_6_C
31	HETLOSS_NA	TCGA-UF-A719-01	CUTCH_p_TCGAb_355_37_52_NSP_GenomeWideSNP_6_G0
50	HETLOSS_NA	TCGA-HD-7229-01	PEAKY_p_TCGA_b164_SNP_N_GenomeWideSNP_6_B12_863
77	NA_HOMDEL	TCGA-BB-4224-01	BALMS_p_TCGAb54and67_SNP_N_GenomeWideSNP_6_A01
78	NA_HOMDEL	TCGA-CV-5434-01	FISTS_p_TCGA_b107_121_SNP_N_GenomeWideSNP_6_F03_
81	NA_HOMDEL	TCGA-P3-A5QA-01	LEGIT_p_TCGA_300_301_302_N_GenomeWideSNP_6_F06_13



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