MVisAGe Vignette

Vonn Walter

1 Introduction

High-profile studies such as those conducted by The Cancer Genome Atlas (TCGA) have provided biomedical researchers unprecedented access to high-quality genomic datasets. Unfortunately, even basic analyses can be difficult for researchers without specialized bioinformatics skills. MVisAGe was designed with this audience in mind, and the package allows users to easily perform bivariate analyses involving mRNA expression and another quantitative genomic variable (e.g. DNA copy number data). As currently implemented, MVisAGe does not perform any sort of hypothesis testing. Instead, MVisAGe allows users to compute and visualize Pearson correlation coefficients on a regional or genomewide basis with the goal of assessing the effect of a genomic alteration (e.g. DNA copy number change) on gene expression.

Although numerous approaches have been developed for bivariate analyses of specific types of genomic data (Huang et al. (2012) and Lahti et al. (2013)), Pearson and Spearman correlation coefficients continue to be widely used, particularly for exploratory analyses. MVisAGe uses Pearson correlation coefficients because they can be computed efficiently with matrix-based approaches. In addition, MVisAGe can produce graphical output for visualizing the correlation coefficients and thus facilitate the identification of regions where genomic alterations (e.g. DNA copy number changes) have an effect on gene expression.

A typical MVisAGe analysis starts with two matrices of quantitative genomic data, one of which contains gene expression data (exp.mat). The second matrix (called cn.mat here) contains data from another genomic variable that may be associated with gene expression, say DNA copy number data. Although MVisAGe may be applied if cn.mat is a matrix of gene-level DNA methylation data, in this vignette we will focus exclusively on DNA copy number data. The rows of both exp.mat and cn.mat the rows are indexed by genes, and the columns are indexed by samples. It is important to note that MVisAGe does not perform any type of preprocessing or normalization. Thus expression measurements should be quantified using RPKM, RSEM, etc., and DNA copy number measurments should be $\log_2$ ratios.

Users can apply MVisAGe to their own data or data that has been downloaded from public repositories. In this vignette we use two TCGA head and neck squamous cell carcinoma (HNSC) datasets that were downloaded from the Broad Institute's Firehose GDAC (https://gdac.broadinstitute.org/). The R data object MVisAGe.RData that is included in the package contains gene expression and DNA copy number data that was produced as follows:

- The gene expression file

  HNSC.rnaseqv2__illuminahiseq_rnaseqv2__unc_edu__Level_3__RSEM_genes_normalized__data.data.txt and the copy number file all_data_by_genes.txt were downloaded,

- We restricted both datasets to rows corresponding to genes in chr11 and chr12,
- We restricted both datasets to the samples in the first 100 columns.

Now we install the package and load the data.

```
> library(MVisAGe)
> data(MVisAGe)
> ls()
```

```
character(0)
```

```
> exp.mat[1:5, 1:5]
```

```
              TCGA.4P.AA8J.01A.11R.A39I.07 TCGA.BA.4074.01A.01R.1436.07
gene_id       "normalized_count"           "normalized_count"
A2ML1|144568 "2258.5118"                   "237.0097"
A2M|2        "8160.1109"                   "1567.7058"
AAAS|8086    "1064.8590"                   "574.6041"
AACS|65985   "1416.7309"                   "580.7702"
              TCGA.BA.4075.01A.01R.1436.07 TCGA.BA.4076.01A.01R.1436.07
gene_id       "normalized_count"           "normalized_count"
A2ML1|144568 "44.4861"                     "6187.5902"
A2M|2        "993.1288"                    "1194.8052"
AAAS|8086    "580.9996"                    "575.6264"
AACS|65985   "1157.7114"                   "2010.7569"
              TCGA.BA.4077.01B.01R.1436.07
gene_id       "normalized_count"
A2ML1|144568 "2346.0058"
A2M|2        "1963.4216"
AAAS|8086    "860.9240"
AACS|65985   "1442.2522"
```

```
> cn.mat[1:5, 1:5]
```

```
      Locus.ID    Cytoband   TCGA.4P.AA8J.01A.11D.A390.01
A2M   "        2" "12p13.31" " 0.184"
A2ML1 "   144568" "12p13.31" " 0.184"
A2MP1 "    -3926" "12p13.31" " 0.184"
AAAS  "     8086" "12q13.13" " 0.022"
AACS  "    65985" "12q24.31" " 0.022"
      TCGA.BA.4074.01A.01D.1432.01 TCGA.BA.4075.01A.01D.1432.01
A2M   " 0.001"                     "-0.063"
A2ML1 " 0.001"                     "-0.063"
A2MP1 " 0.001"                     "-0.063"
AAAS  " 0.013"                     "-0.140"
AACS  " 0.002"                     "-0.234"
```

The above output shows that exp.mat and cn.mat are matrices of character values that contain extraneous rows and columns. Moreover, the gene names are not the same in the two matrices, and neither are the sample identifiers. The section below introduces two 'helper functions' that are specifically designed to reformat TCGA data downloaded from the Broad Institute's Firehose GDAC. Users with comparable gene names and sample identifiers may choose to skip ahead to the following section.

2 Reformatting TCGA Data

The tcga.exp.convert() and tcga.cn.convert() functions can be applied to reformat exp.mat and cn.mat, respectively. exp.mat = tcga.exp.convert(exp.mat) removes the extraneous top row, shortens the TCGA barcodes in the column names to the form TCGA.XX.XXXX.XXX, rewrites the row names of the form GENE_SYMBOL|GENE_ID_number as GENE_SYMBOL, removes any rows corresponding to duplicate gene symbols, and creates a numeric matrix.

```
> exp.mat = tcga.exp.convert(exp.mat)
> exp.mat[1:5, 1:5]
```

```
         TCGA.4P.AA8J.01A TCGA.BA.4074.01A TCGA.BA.4075.01A TCGA.BA.4076.01A
A2ML1           2258.5118         237.0097          44.4861        6187.5902
A2M             8160.1109        1567.7058         993.1288        1194.8052
AAAS            1064.8590         574.6041         580.9996         575.6264
AACS            1416.7309         580.7702        1157.7114        2010.7569
AASDHPPT         518.8723        1070.5634        1872.1694         584.5468
         TCGA.BA.4077.01B
A2ML1           2346.0058
A2M             1963.4216
AAAS             860.9240
AACS            1442.2522
AASDHPPT         452.8393
```

cn.mat = tcga.cn.convert(cn.mat) removes extraneous columns, shortens TCGA barcodes in the column names to the form TCGA.XX.XXXX.XXX, and creates a numeric matrix.

```
> cn.mat = tcga.cn.convert(cn.mat)
> cn.mat[1:5, 1:5]
```

```
      TCGA.4P.AA8J.01A TCGA.BA.4074.01A TCGA.BA.4075.01A TCGA.BA.4076.01A
A2M              0.184            0.001           -0.063            0.000
A2ML1            0.184            0.001           -0.063            0.000
A2MP1            0.184            0.001           -0.063            0.000
AAAS             0.022            0.013           -0.140            0.000
AACS             0.022            0.002           -0.234           -0.032
```

```
      TCGA.BA.4077.01B
A2M             -0.708
A2ML1           -0.262
A2MP1           -0.708
AAAS            -0.011
AACS            -0.016
```

At this point both exp.mat and cn.mat are numeric matrices whose row names are gene symbols and whose column names are TCGA barcodes.

3 Preparing Data for Analysis

A gene annotation file (gene.annot) containing the chromosome number, genomic position, and cytoband information is needed in order to plot gene-level Pearson correlation coefficients. One such file is included here based on HGNC gene symbols and hg38 gene positions, but users can easily create their own gene.annot files if they have different gene identifiers or different genome builds. Gene annotation files should contain the following three columns: column 1 = chromosome number written as ”chr1”, column 2 = genomic position, column 3 = cytoband. The row names of gene.annot should be the same type of gene names that are used in exp.mat and cn.mat. An example is shown below:

```
> head(gene.annot)

         chr     pos          cytoband
A1BG     "chr19" " 58350152" "q13.43"
A1BG-AS1 "chr19" " 58353576" "q13.43"
A1CF     "chr10" " 50842541" "q11.23"
A2M      "chr12" "  9091834" "p13.31"
A2M-AS1  "chr12" "  9066615" "p13.31"
A2ML1    "chr12" "  8855259" "p13.31"
```

Optional sample annotation files (sample.annot) are two column files that contain categorical sample annotation data about the samples, e.g. disease status (tumor/normal) or vital status (alive/dead). The first column contains sample ids that must have the same form as the column names of exp.mat and cn.mat, while the second column contains the categorical sample annotation data. In the example below we will use human papillomavirus (HPV) infection status (HPV+/HPV-) from the TCGA HNSC manuscript (Nature, 2015).

```
> head(sample.annot)

     Barcode            New.HPV.Status
[1,] "TCGA.BA.4074.01A" "HPV-"
[2,] "TCGA.BA.4076.01A" "HPV-"
[3,] "TCGA.BA.4077.01A" "HPV+"
```

```
[4,] "TCGA.BA.4078.01A" "HPV-"
[5,] "TCGA.BA.5149.01A" "HPV-"
[6,] "TCGA.BA.5151.01A" "HPV-"
```

It is often the case that different genes appear in gene.annot, exp.mat, and cn.mat. Moreover, the sample ids in exp.mat and cn.mat need not be the same. For this reason MVisAGe includes two additional 'helper functions' that are useful for preparing data for analysis. prepped.data = data.prep(exp.mat, cn.mat, gene.annot, sample.annot, log.exp = F) produces a list containing four elements, and the names are "exp", "cn", "gene.annot", and "sample.annot". Gene names and sample ids now appear in the same order. The default option sample.annot = NULL should be used if no sample annotation data is available. The final argument, log.exp, specifies whether the expression data has been log transformed. The default is FALSE, and in this case a $\log_2(x+1)$ transformation is applied to exp.mat.

```
> prepped.data = data.prep(exp.mat, cn.mat, gene.annot, sample.annot, log.exp = F)
```

```
[1] "Checking gene names"
[1] "Gene names in expression vs. CN"
[1] TRUE
[1] "Gene names in expression vs. gene annotation"
[1] TRUE
[1] "Checking sample names"
[1] "Sample names in expression vs. CN"
[1] TRUE
[1] "Sample names in expression sample annotation"
[1] TRUE
```

```
> prepped.data[["exp"]][1:5, 1:5]
```

```
          TCGA.BA.4074.01A TCGA.BA.4076.01A TCGA.BA.4078.01A TCGA.BA.5149.01A
A2M              10.615359        10.223767        12.156678        11.497423
A2ML1             7.894877        12.595395         9.854848         7.521612
AAAS              9.168933         9.171493        10.005123        10.033737
AACS              9.184306        10.974240        10.504708         9.861332
AASDHPPT         10.065501         9.193641         9.012680         8.307791
          TCGA.BA.5151.01A
A2M              12.837524
A2ML1            13.287780
AAAS              8.865353
AACS             10.649798
AASDHPPT         10.203820
```

```
> prepped.data[["cn"]][1:5, 1:5]
```

```
          TCGA.BA.4074.01A TCGA.BA.4076.01A TCGA.BA.4078.01A TCGA.BA.5149.01A
A2M                  0.001            0.000            0.063           -0.351
```

```
A2ML1               0.001            0.000            0.063            -0.351
AAAS                0.013            0.000            0.070             0.014
AACS                0.002           -0.032            0.043             0.014
AASDHPPT           -0.481           -0.806           -0.758            -0.752
         TCGA.BA.5151.01A
A2M                -0.002
A2ML1              -0.002
AAAS               -0.002
AACS               -0.003
AASDHPPT            0.000
```

```
> head(prepped.data[["gene.annot"]])
```

```
         chr  pos         cytoband
A2M      "12" "  9091834" "p13.31"
A2ML1    "12" "  8855259" "p13.31"
AAAS     "12" " 53314541" "q13.13"
AACS     "12" "125104351" "q24.31"
AASDHPPT "11" "106088128" "q22.3"
ABCB9    "12" "122946499" "q24.31"
```

```
> head(prepped.data[["sample.annot"]])
```

```
     Barcode            New.HPV.Status
[1,] "TCGA.BA.4074.01A" "HPV-"
[2,] "TCGA.BA.4076.01A" "HPV-"
[3,] "TCGA.BA.4078.01A" "HPV-"
[4,] "TCGA.BA.5149.01A" "HPV-"
[5,] "TCGA.BA.5151.01A" "HPV-"
[6,] "TCGA.BA.5152.01A" "HPV-"
```

4 Computing correlation coefficients

The corr.compute() function computes gene-level Pearson correlation coefficients based on input matrices of gene expression and DNA copy number data with matched genes and sample ids. This function is called by corr.list.compute(), which uses the output of data.prep() as input. corr.list.compute(exp.mat, cn.mat, gene.annot, sample.annot) is also a list, and its length is equal to the number of distinct values of the categorical sample annotation data (the length is 1 if sample.annot = NULL). Each list member is a five-column matrix whose rows are indexed by genes. Column 1 = chromosome number, column 2 = genomic position (in base pairs), column 3 = cytoband, column 4 = the gene-level Pearson correlation coefficient computed from the expression and copy number data, and column 5 = the squared Pearson correlation coefficient. If sample annotation is provided, the correlation coefficients are computed separately using the samples that correspond to each unique value of the categorical sample annotation data. In the example below the Pearson correlation coefficients are shown for the samples in the HPV- samples in the TCGA HNSC cohort.

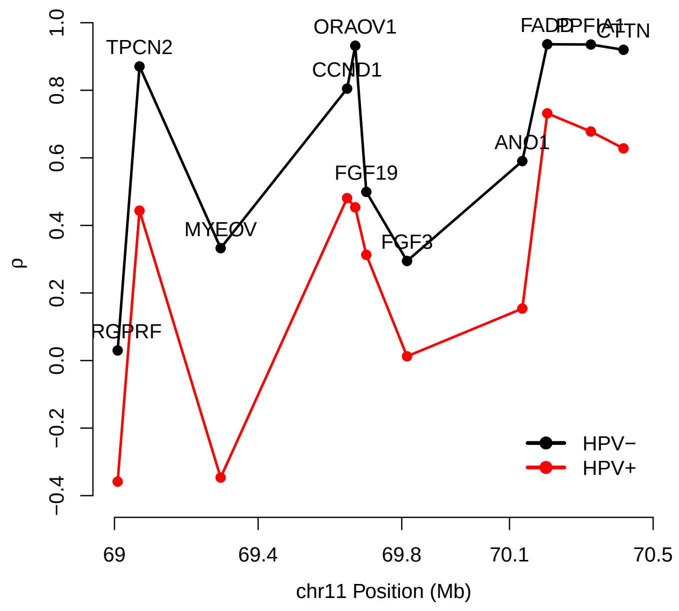
```
> output.list = corr.list.compute(prepped.data[["exp"]], prepped.data[["cn"]], prepped.data[
> names(output.list)

[1] "HPV-" "HPV+"

> head(output.list[["HPV-"]])

         chr  pos         cytoband R                     R^2
A2M      "12" "  9091834" "p13.31" "0.087134562922435"   "0.00759243205568379"
A2ML1    "12" "  8855259" "p13.31" "-0.0104125927538568" "0.00010842208785767"
AAAS     "12" " 53314541" "q13.13" "0.544343099198941"   "0.296309409645508"
AACS     "12" "125104351" "q24.31" "0.573088639646666"   "0.328430588892066"
AASDHPPT "11" "106088128" "q22.3"  "0.901481313083607"   "0.812668557838944"
ABCB9    "12" "122946499" "q24.31" "0.529611347206305"   "0.280488179089677"
```

5 Plotting correlation coefficients

As noted in the introduction, MVisAGe can produce graphical output to visualize the Pearson correlation coefficients produced by corr.list.compute() in a given genomic region. Three different functions are available depending on the size of the genomic region of interest. In each case the input is a list produced by corr.list.compute(), and there are a variety of graphical parameters that can be defined by the user.

As name implies, unsmooth.region.plot() plots raw Pearson correlation coefficients in a region defined by the user. Because of the inherent noise, this function is best suited to regions containing a relatively small number of genes.

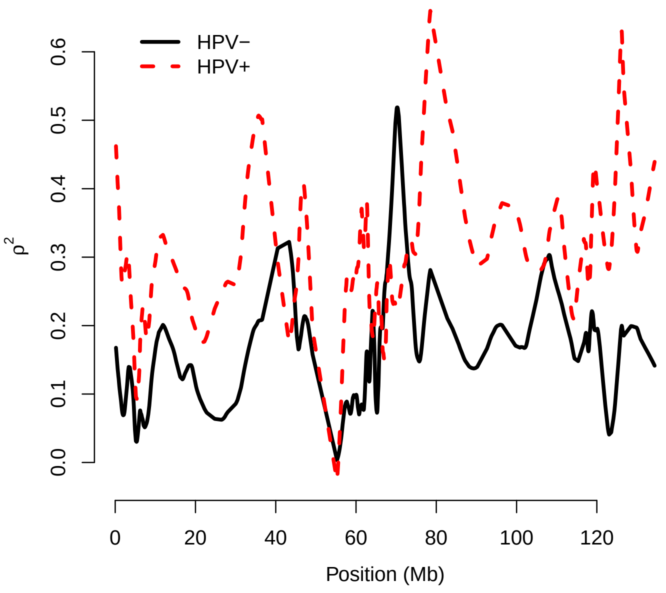
```
> unsmooth.region.plot(
+         plot.list = output.list,
+         plot.chr = 11,
+         plot.start = 69e6,
+         plot.stop = 70.5e6,
+         plot.column = "R",
+         plot.points = T,
+         plot.lines = T,
+         gene.names = T,
+         annot.colors = c("black", "red", "green", "blue", "cyan"),
+         vert.pad = .05,
+         num.ticks = 5,
+         ylim.low = NULL,
+         ylim.high = NULL,
+         pch.vec = c(19, 19),
+          lty.vec = NULL,
+          lwd.vec = c(2, 2),
+          plot.legend = T,
+          legend.loc = "bottomright"
+          )
```



The above figure shows that HPV- samples have markedly higher Pearson correlation coefficients in chr11q13.3, a region that is frequently amplified in HNSC and contains the known driver genes *CCND1, FADD*, and *CTTN*.

The smooth.region.plot() function applies loess smoothing to the Pearson correlation coefficients so users can visualize regional trends in the Pearson correlation coefficients over larger chromosomal regions. This is done because the noise present in the gene-level Pearson correlation coefficients can make it difficult to observe regional trends that may be of interest. The level of smoothing is controlled by the loess.span parameter, which is measured in number of genes. In particular, loess.span is used to define the *span* parameter in the loess function using the formula $span = \frac{\text{loess.span}}{n+(2*\text{expand.size})}$, where n is the number of genes in the region and expand.size is another parameter (also measured in number of genes) used to lessen the effects of smoothing at the end of the region. Additional information about the *span* parameter can be found in help menu for the loess() function, while details about expand.size are available in the forthcoming manuscript with Walter et al.

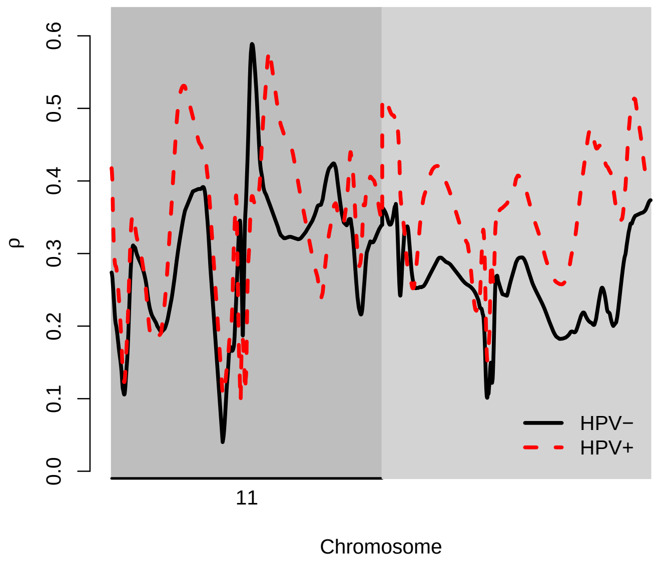
```
> smooth.region.plot(
+         plot.list = output.list,
+         plot.chr = 11,
+         plot.start = 0e6,
+         plot.stop = 135e6,
+         plot.column = "R^2",
+         annot.colors = c("black", "red", "green", "blue", "cyan"),
+         vert.pad = 0,
+         ylim.low = NULL,
+         ylim.high = NULL,
+         lty.vec = c(1, 2),
+         lwd.vec = c(3, 3),
+         plot.legend = T,
+         legend.loc = "topleft",
+         loess.span = 50,
+         expand.size = 3,
+         xaxis.label = "Position (Mb)",
+         yaxis.label = expression(rho^2),
+         main.label = NULL,
+         axis.cex = 1,
+         label.cex = 1,
+         xaxis.line = 2.5,
+         yaxis.line = 2.5,
+         main.line = 0
+         )
```



The above figure shows that HPV- samples exhibit a pronounced peak near 70Mb that is effectively absent in the HPV+ samples, which agrees with the output from unsmooth.region.plot() shown above.

Although the smooth.region.plot() function is useful for plotting smoothed Pearson correlation coefficients in a region of a given chromosome, smooth.genome.plot() should be used to make similar figures for multiple chromosomes or across the genome. The figure produced in the following example is similar to the one produced above by smooth.region.plot(), only now we show both chr11 and chr12.

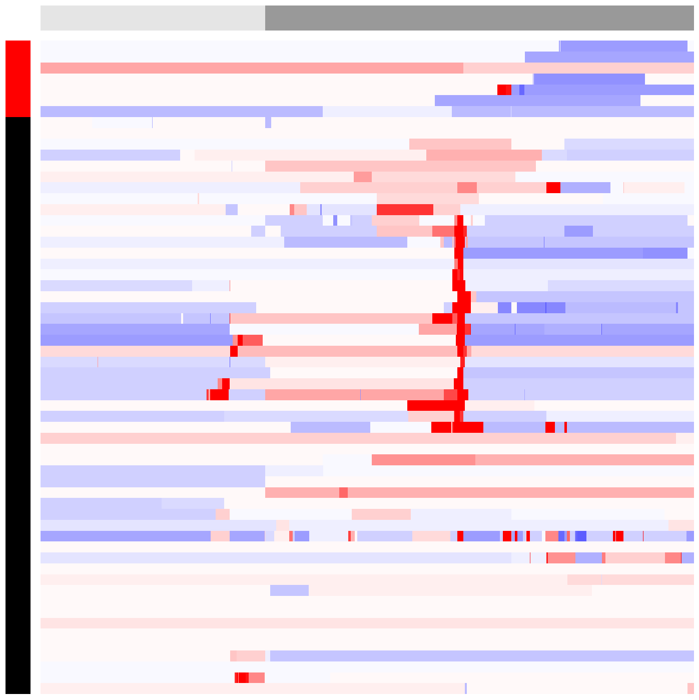
```
> smooth.genome.plot(plot.list = output.list,
+         plot.column = "R",
+         annot.colors = c("black", "red", "green", "blue", "cyan"),
+         vert.pad = 0.05,
+         ylim.low = NULL,
+         ylim.high = NULL,
+         plot.legend = T,
+         legend.loc = "bottomright",
+         lwd.vec = c(3, 3),
+         lty.vec = c(1, 2),
+         loess.span = 100,
+         expand.size = 100,
+         rect.colors = c("light gray", "gray"),
+         chr.label = T,
+           xaxis.label = "Chromosome",
+           yaxis.label = expression(rho),
+           main.label = NULL,
+           axis.cex = 1,
+           label.cex = 1,
+           xaxis.line = 1.5,
+           yaxis.line = 2.5,
+           main.line = 0)
```



6 Copy number heatmaps

Recurrent DNA copy number changes are of interest because may be associated with changes in expression of regional genes. Although plots of the Pearson correlation coefficients can be used to visualize the magnitude of the association in a given region, copy number heatmaps are useful because they can be used to visualize the DNA copy number changes. Therefore the copy number heatmaps produced by cn.region.heatmap() can be used in conjunction with plots produced by smooth.region.plot() to explore copy number changes and the resulting effect on gene expression in a given chromosomal region.

```
> cn.region.heatmap(cn.mat = prepped.data[["cn"]],
+           gene.annot = prepped.data[["gene.annot"]],
+           plot.chr = 11,
+           plot.start = 0e6,
+           plot.stop = 135e6,
+           sample.annot = prepped.data[["sample.annot"]],
+           sample.cluster = T,
+           low.thresh = -2,
+           high.thresh = 2,
+           num.cols = 50,
+           collist = c("blue", "white", "red"),
+           annot.colors = c("black", "red"),
+           plot.list = output.list,
+           plot.sample.annot = T,
+           cytoband.colors = c("gray90", "gray60")
+           )
```



The above heatmap illustrates the presence of recurrent copy number gains at chr11q13.3 in the HPV- samples (black annotation bars) that are largely absent in the HPV+ samples (red annotation bars). The output from unsmooth.region.plot() and smooth.region.plot() shown above suggests that these amplifications lead to increased expression of regional genes, thereby illustrating their importance.

7 References

Huang N, Shah PK, Li C (2012). Lessons from a decade of integrating cancer copy number alterations with gene expression profiles. Brief. Bioinform. 13(3): 305 - 316.
Lahti L, Schafer M, Klein H-U, Bicciato S, Dugas M (2013). Cancer gene prioritization by integrative analysis of mRNA expression and DNA copy number data: a comparative review. Brief. Bioinform. 14(1): 27 - 35.
The Cancer Genome Atlas Research Network (2015). Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 517(7536) 576 - 582.