

Data cleanup and summary statistics with R

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Getting started

This tutorial assumes you know how to load data into an RStudio session, view a dataframe and explore columns/rows of a dataframe in R. Knowing how to visualize data as scatterplots will also be helpful, though not essential.

We will be using two packages - reshape2 and ggpubr.

- reshape2 is a package with 2 main functions, `melt` and `dcast`. It is helpful for flexibly reshaping your data.
- ggpubr is a graphing package, that lets you create publication ready ggplots, and automatically add significance levels to your figures.

Let us also load up ggplot2 into our current environment, just in case we want to make pretty plots.

```
# If you don't have a package installed already install.packages('packagename')  
# Otherwise, load it into the environment  
library(ggplot2)  
library(ggpubr)  
library(reshape2)
```

R provides excellent support for statistical analysis. The data we will be working with is cell-line expression data from the LINCS1000 dataset. I have adapted this dataset for our use, which is available at the same spot you found this tutorial. You can also download the original data from [here](#).

Cleaning my data

```
## Load the data
data_df <- read.table("data_sp_scaled.txt", sep = "\t", stringsAsFactors = F, header = T)
## and the covariate information
metadata_df <- read.table("metadata.txt", sep = "\t", stringsAsFactors = F, header = T)
```

As always, let us start by figuring out what we are working with. The `dim()` function prints the dimensions of a dataframe, and `head()` function shows the first 6 rows of a dataframe. You can also print only the row-count (or column-count) with the commands `nrow()` and `ncol()`.

The command `dim(data_df)` tells us that the data has 35 rows and 33842 columns. Similarly, the row count and column count values for the metadata dataframe, `metadata_df`, are 35, 5, respectively.

Notice that if we try to print the first 6 rows of `data_df`, the output is immense. Thus, instead of `head(data_df)`, we will print the first 6 rows, with the first 10 columns. We can select these columns with `data_df[,1:10]`.

	TSPAN6	TNMD	DPM1	SCYL3	C1orf112	FGR	CFH	FUCA2	GCLC	NE
HCC1806	3.505880	0	NA	NA	3.534551	NA	2.8251175	5.727614	NA	4.600
MCF10A	4.027427	0	5.549405	2.482252	3.963324	0.0000000	5.2812262	5.292880	6.521847	4.172
SKBR3	2.684550	0	6.747064	3.199656	4.189142	0.1014213	0.0096204	5.240616	4.322539	3.518
HS578T	NA	0	NA	1.424340	3.363768	0.0369093	5.2759113	5.841921	3.851815	4.461
MDAMB231	4.287758	NA	5.693864	2.217807	4.619957	0.0000000	0.9724045	5.677292	4.046207	5.442
BT20	3.335776	0	6.602087	2.648148	3.904788	0.0000000	0.1888233	4.781958	5.623793	4.138

The column names correspond to genes, and the rows represent samples. These sample names correspond to the column `c1_id` in `metadata_df` (can you quickly verify this using `head()`?).

We can also quickly ensure we don't have random unexpected values (such as characters or alphabets where we expect numbers) using the query `is.numeric`. You can quickly check what this function does, by entering `?is.numeric` in your R prompt.

```
table(sapply(data_df, is.numeric))
```

```
##
## TRUE
## 33842
```

Looks like all our columns are numeric!

Take a quick look at the output from the `head()` function a couple of lines ago. It looks like we have some missing values in our data! Before we try and figure out a fix for this, let us calculate how many genes have missing values, or if the problem is only in a single sample.

R has a handy command, `complete.cases`, for checking if there are any rows containing missing values. It returns a TRUE/FALSE value for every row. We can summarize the results of this **list** in tabular form, using the function `table()`.

```
table(complete.cases(data_df))
```

Var1	Freq
FALSE	5
TRUE	30

It appears 5 samples have atleast 1 gene with a missing value. We can redo this test for the genes, after **transposing** our data. This is done using the function `t()`.

```
table(complete.cases(t(data_df)))
```

Var1	Freq
FALSE	18941
TRUE	14901

Dealing with missing data

Over 50% of the genes across 5 samples are missing. We can deal with this either using imputation strategies, or by discarding the problematic samples. As imputation strategies are an entire discussion by themselves, we will *not* dive into them today (additional resources available at end of tutorial). Instead, we will take the easy way out and remove the samples with NAs. Good thing we have already covered a quick way to unselect these samples!

```
data_df_clean <- data_df[complete.cases(data_df), ]
```

If you were perusing the previous tutorial, you would have noticed us using `na.omit` to find rows in a dataframe that contain any NA value (in any column). These two commands are functionally the same, but `complete.cases` can be used on a subset of columns instead of the entire dataframe as well. For example, if we wanted only to remove the samples where 1 or more of certain genes were missing, we could have chosen them with `data_df[complete.cases(data_df[,c("myFavGene1", "myFavGene2", ..., "myfavGeneN")]),]`. Don't forget the comma after the row-selection!

After this, we have 30 samples and 33842 genes. We can do a quick 'smell-test' on this data, by using the dataframe function `summary()`. This function calculates summary statistics for each column in the dataframe. We can transpose the dataframe so that the samples become columns (instead of rows).

```
summary(t(data_df_clean))
```

BT20	MCF7	PDX1258	PDX1328	BT549	HCC38	HCC
Min. : 0.0000	Min. : 0.0000	Min. : 0.0000	Min. : 1.000	Min. : 0.0000	Min. : 0.0000	Min. : 0.0000
1st Qu.: 0.0000	1st Qu.: 0.0000	1st Qu.: 0.0000	1st Qu.: 1.000	1st Qu.: 0.0000	1st Qu.: 0.0000	1st Qu.: 0.0000
Median : 0.1565	Median : 0.2083	Median : 0.3089	Median : 1.123	Median : 0.1346	Median : 0.2004	Median : 0.2004
Mean : 1.6150	Mean : 1.6330	Mean : 1.7137	Mean : 3.433	Mean : 1.5792	Mean : 1.6433	Mean : 1.6433
3rd Qu.: 3.2142	3rd Qu.: 3.2164	3rd Qu.: 3.2897	3rd Qu.: 3.648	3rd Qu.: 3.0660	3rd Qu.: 3.1915	3rd Qu.: 3.1915
Max. :13.9749	Max. :13.5385	Max. :400.7026	Max. :310.614	Max. :12.7921	Max. :12.9747	Max. :12.9747

Well, its still hard to read! Enter ggplot! However, we need to set up our data such that we can pass in a column with the sample name, and a column with the values being plotted.

For this, we will use the `melt` function from the **reshape2** package. The melt function is helpful in converting your data from the *long* to *wide* format. A similar function, `cast()`, can be used when you wish to calculate

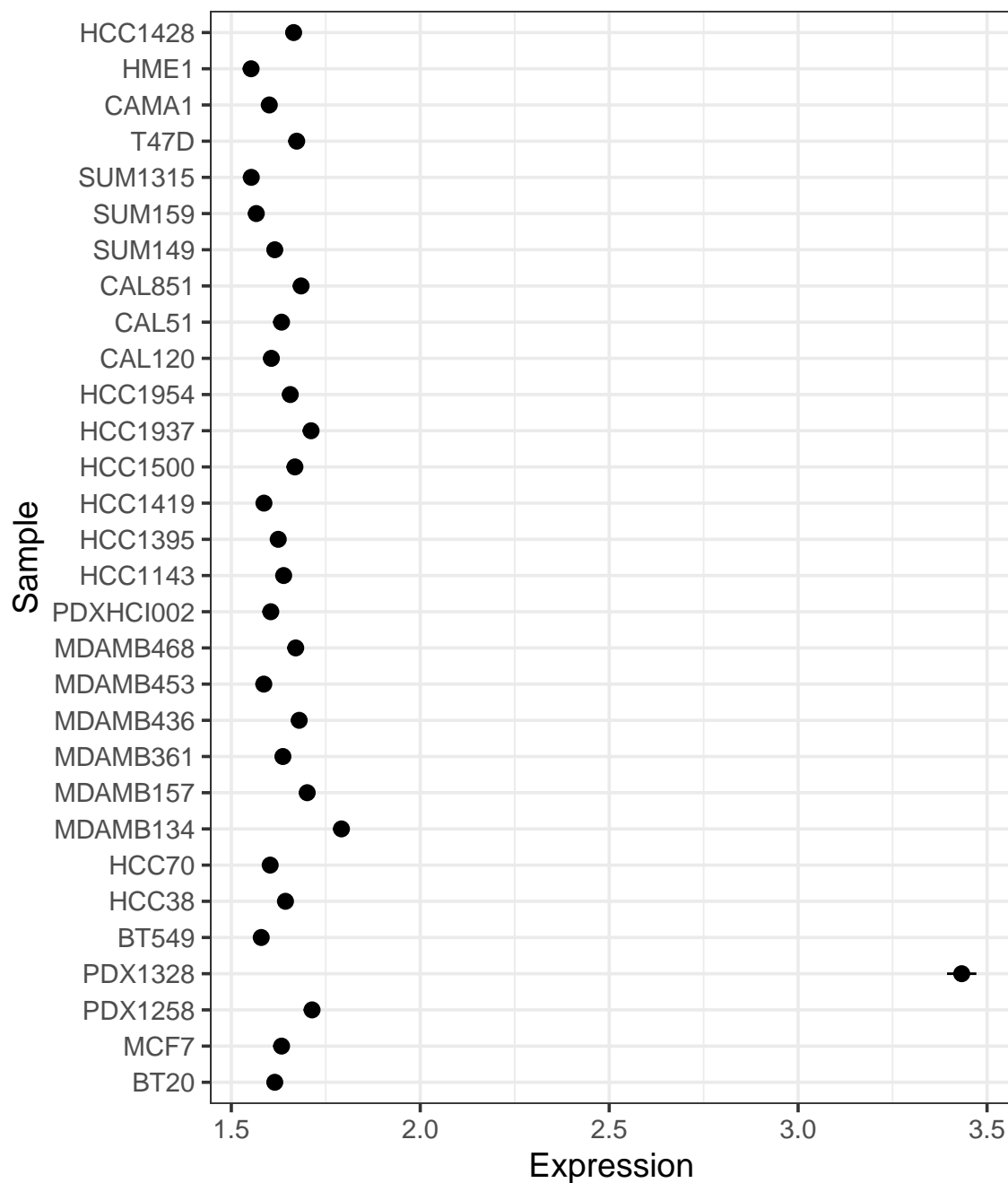
summary statistics on your data.

```
data_compact_df = melt(t(data_df_clean))  
colnames(data_compact_df) = c("Gene", "Sample", "Expression")
```

Dealing with outliers

ggplot has a handy `geom_object` (remember these from the ggplot tutorial?) for summary statistics. The `stat_summary()` (or `geom_summary()`) method allows us to plot a pointrange plot showing the mean and 2 x standard deviation.

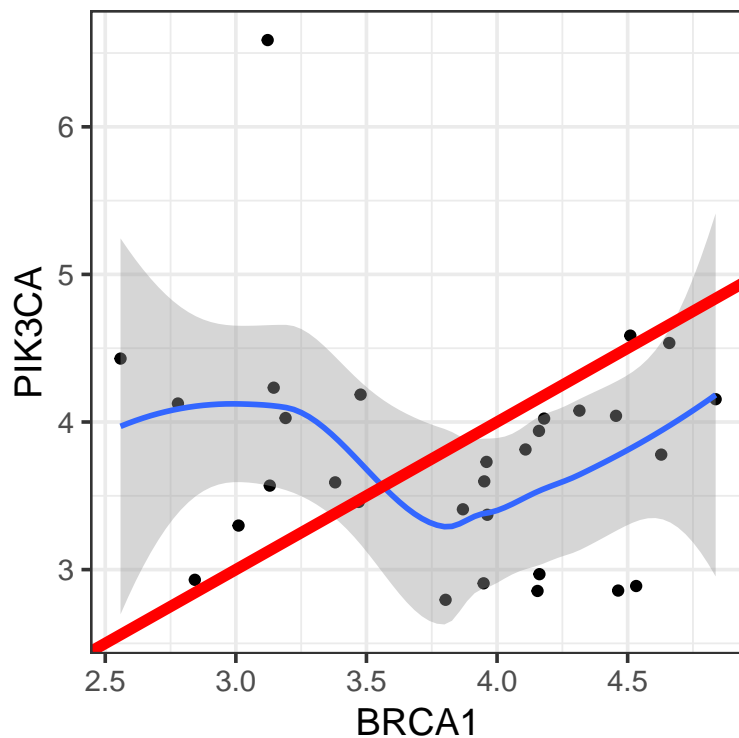
```
## stat_summary() defaults to the categories defined on the x axis, and summarizes
## the numeric spread on the y-axis We use coord_flip() to switch the categories
## to the y-axis, to improve readability
ggplot(data_compact_df, aes(x = Sample, y = Expression)) + stat_summary() + theme_bw(base_size = 14) +
  coord_flip()
```



```
## stat_summary with 1 Standard Deviation around mean
ggplot(data_compact_df, aes(x = Sample, y = Expression)) + stat_summary(fun.args = list(mult = 1)) +
  theme_bw(base_size = 14) + coord_flip()
```

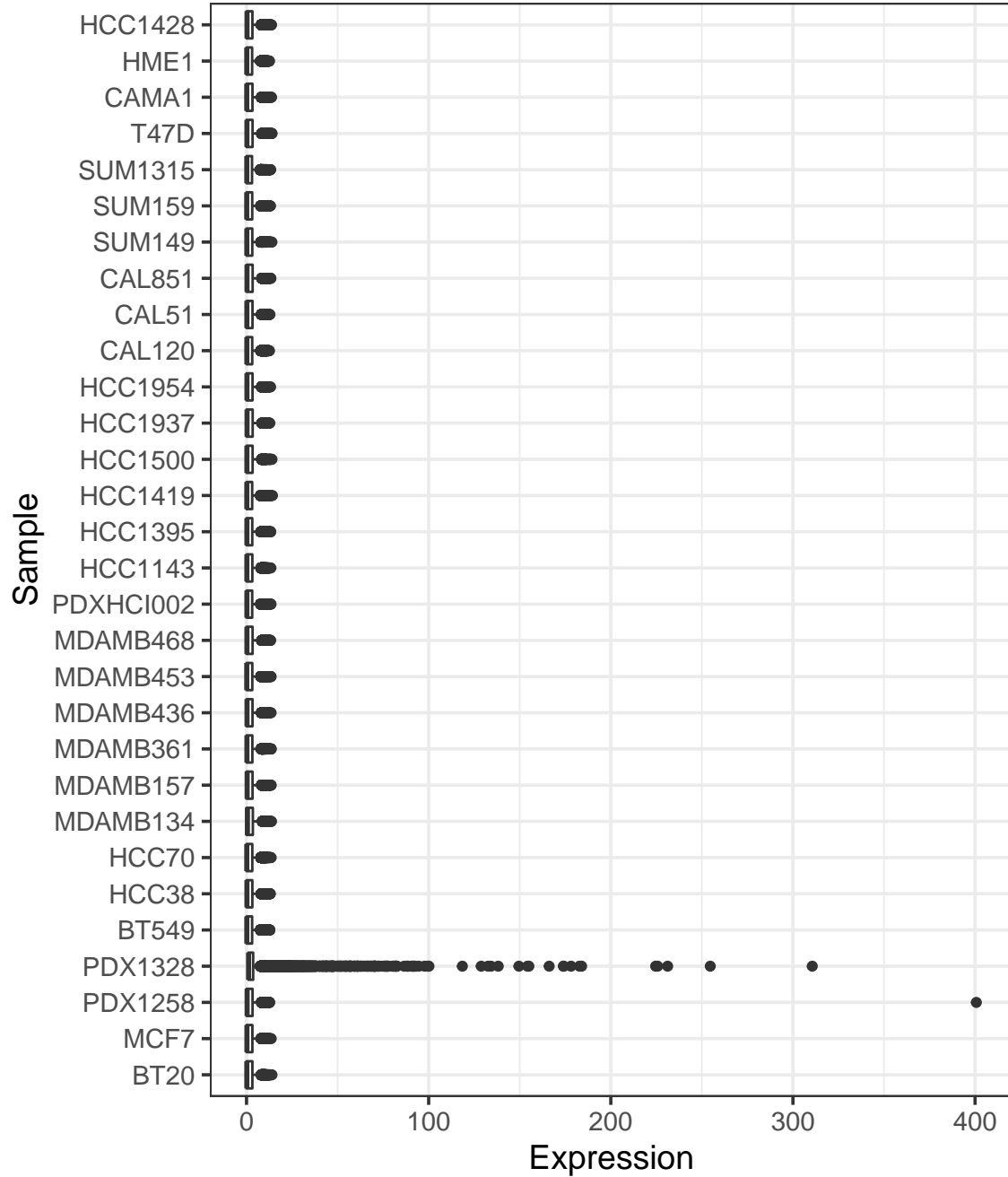
Segue: Another ggplot method is `stat_smooth()` (or `geom_smooth()`). This is helpful for plotting a line of best fit on your data. When you may want to compare this with a standard straight line, `geom_abline` is quite helpful.

```
## geom_point to visualize the scatterplot stat_smooth to fit the blue line with
## confidence intervals in grey geom_abline fits an x=y line by default
## (intercept=0, slope=1).
ggplot(data_df_clean, aes(x = BRCA1, y = PIK3CA)) + geom_point() + stat_smooth() +
  geom_abline(colour = "red", size = 2) + theme_bw(base_size = 14)
```



End of Segue: Looking back at our `stat_summary` figure, we have an anomalous sample! The sample PDX1328 has readings that lie extremely outside the range of the rest of the samples. We can see this more clearly with a boxplot version of the datapoints, plotted using `geom_boxplot()`.

```
ggplot(data_compact_df, aes(x = Sample, y = Expression)) + geom_boxplot() + theme_bw(base_size = 14) + coord_flip()
```

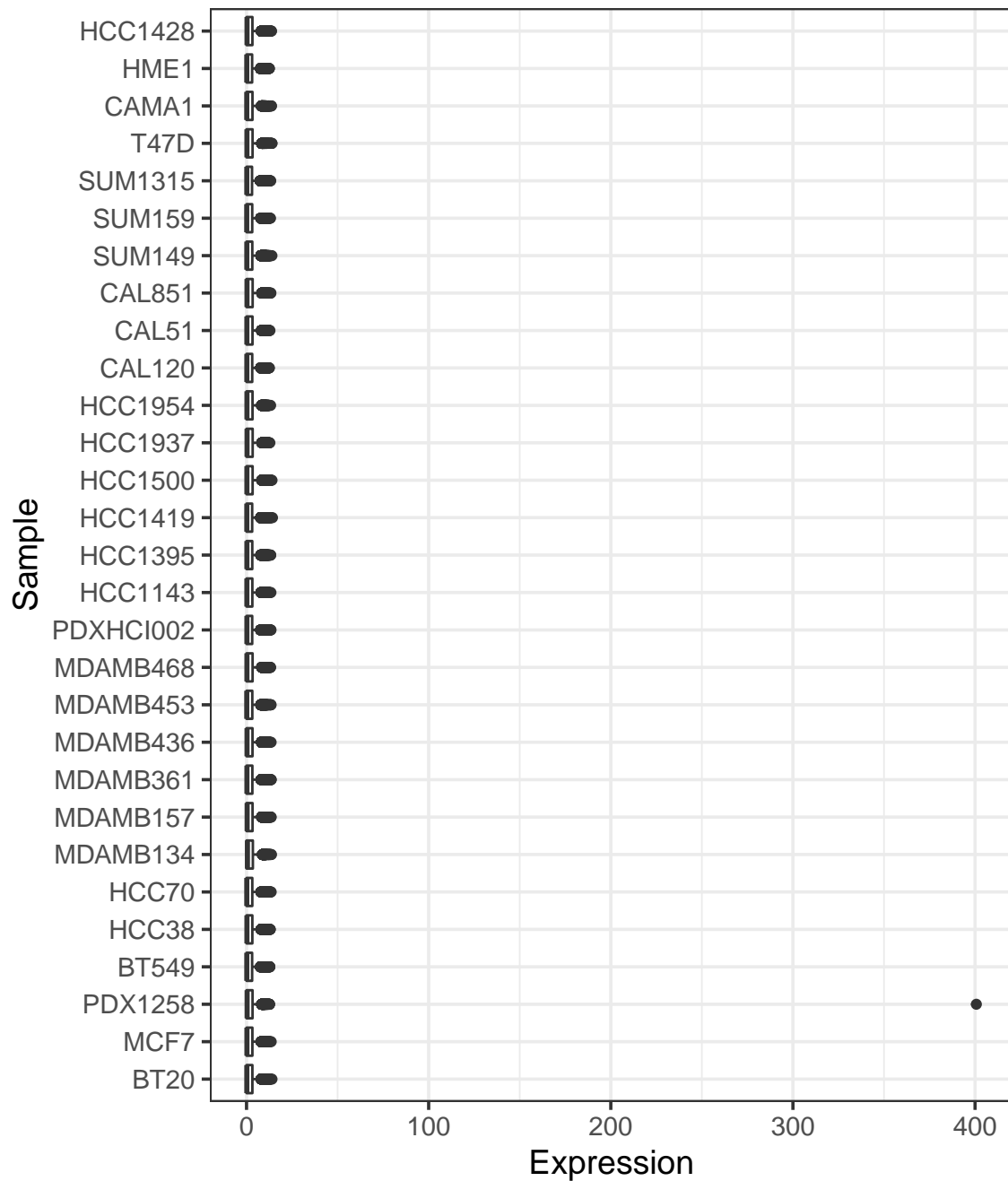


We can remove the outlier sample using the following command (note that we are making changes to the sample x gene dataframe, not the melted version). For the sake of this tutorial, we have already decided that this is a **technical outlier**, whereby the readings themselves are in an entirely different range from the rest of the data. Had it been a biological outlier, you may want to keep it in your data!

```
data_df_clean2 = data_df_clean[!(rownames(data_df_clean) %in% c("PDX1328")), ]
```

Alright, so what does the data look like after that?

```
# First we melt our data
data_compact_df = melt(t(data_df_clean2))
colnames(data_compact_df) = c("Gene", "Sample", "Expression")
# Then we plot it
ggplot(data_compact_df, aes(x = Sample, y = Expression)) + geom_boxplot() + theme_bw(base_size = 14) +
  coord_flip()
```

Also note that while this was easy to do for a small set of samples, you may not be able to visually identify outliers in large datasets. One handy approach in that case is to generate PCA decompositions of your data, and plot the first 2 principal components. If you see a sample sitting further away from the rest, that's an outlier! There are also more sophisticated approaches for dealing with outliers, explained nicely at [this blogpost](#)

It looks like PDX1258 has an outlier gene. We can easily print out the gene from our melted dataframe, with the command `data_compact_df[data_compact_df$Expression > 300, "Gene"]`. This returns TSPAN6. We can either remove this gene entirely, or replace it with the mean value. Sample metadata information can come in handy at this point. Let us see what information the metadata dataframe can provide:

```
print(metadata_df)
```

cl_id	cl_provider_name	cl_provider_catalog_id
CAL51	Leibniz Institute	ACC-302
MCF7	ATCC	HTB-22
HME1	ATCC	CRL-4010
SKBR3	ATCC	HTB-30
MDAMB231	ATCC	HTB-26
BT20	ATCC	HTB-19
BT549	ATCC	HTB-122
CAMA1	ATCC	HTB-21
HC1143	ATCC	CRL-2321
HCC1395	ATCC	CRL-2324
HCC1419	ATCC	CRL-2326
HCC1428	ATCC	CRL-2327
HCC1806	ATCC	CRL-2335
HCC1937	ATCC	CRL-2336
HCC1954	ATCC	CRL-2338
HCC38	ATCC	CRL-2314
HCC70	ATCC	CRL-2315
HS578T	ATCC	HTB-126
MDAMB134	ATCC	HTB-23
MDAMB157	ATCC	HTB-24
MDAMB361	ATCC	HTB-27
MDAMB436	ATCC	HTB-130
MDAMB453	ATCC	HTB-131
MDAMB468	ATCC	HTB-132
T47D	ATCC	HTB-133
HCC1500	ATCC	CRL-2329
MCF10A	ATCC	CRL-10317
SUM1315	Asterand	SUM-1315MO2
SUM149	Asterand	SUM-149PT
SUM159	Asterand	SUM-159PT
CAL120	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures	ACC-459
CAL851	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures	ACC-440
PDX1258	Dan Stover (Harvard Medical School)	
PDX1328	Caitlin Mills (Harvard Medical School)	
PDXHCI002	Dan Stover (Harvard Medical School)	

PDX1258 is a breast carcinoma. We can see the different disease categories by summarizing the contents of the column `cl_disease_detail`. We can also sort the table while we are at it...

```
sort(table(metadata_df$cl_disease_detail))
```

Var1	Freq
breast fibrocystic disease	1
normal	1
squamous cell carcinoma	1

Var1	Freq
breast medullary carcinoma	2
unknown	3
breast carcinoma	5
breast adenocarcinoma	10
breast ductal carcinoma	12

As there are 5 `breast carcinoma`s in this dataset, we can potentially set the value of PDX1258 to the average value of the gene in other breast carcinomas. If we connect our metadata with our expression data, it will be easy to select the gene and samples of interest. For this we use the `merge` function. Merging requires a column that has the same values in the 2 different dataframes we are joining. Note that we can specify the column using `by="shared column"` if the column has the same name in the 2 dataframes.

```
## Merge expression and metadata
data_merged = merge(data_compact_df, metadata_df, by.x = "Sample", by.y = "cl_id")
## Select PDX1258's outlier gene
brca_tspan_df = data_merged[data_merged$Gene == "TSPAN6" & data_merged$cl_disease_detail ==
  "breast carcinoma", ]
```

	Sample	Gene	Expression	cl_provider_name	cl_provider_catalog_id	cl_cell_line
101527	CAL51	TSPAN6	5.6334571	Leibniz Institute	ACC-302	epithelial
679083	MDAMB453	TSPAN6	0.2958427	ATCC	HTB-131	epithelial
750048	PDX1258	TSPAN6	400.7026020	Dan Stover (Harvard Medical School)		epithelial
913735	T47D	TSPAN6	3.9403834	ATCC	HTB-133	epithelial

Notice that there are only 4 samples here, and CAL51 is similar to PDX1258 as both are epithelial-like cell lines. We can set the TSPAN6 value for PDX1258 the same as sample CAL51, or the average of the 3 breast carcinomas.

```
## Firstly we update data_df_clean2 Notice how we select the row with the sample
## name, and gene with the gene name
data_df_clean2["PDX1258", "TSPAN6"] = mean(brca_tspan_df[brca_tspan_df$Sample !=
  "PDX1258", "Expression"])
## Then we recalculate the melted version of this dataframe
data_compact_df = melt(t(data_df_clean2))
colnames(data_compact_df) = c("Gene", "Sample", "Expression")
```

Comparing groups (and plotting the significance values)

For the last bit, we will use an in-built dataset, `airquality`. You can load it into your current environment by typing `data(airquality)`.

```
data(airquality)
```

Ozone	Solar.R	Wind	Temp	Month	Day
41	190	7.4	67	5	1
36	118	8.0	72	5	2
12	149	12.6	74	5	3
18	313	11.5	62	5	4
NA	NA	14.3	56	5	5
28	NA	14.9	66	5	6

We will use the package `ggpubr`. This package is quite similar to `ggplot`, but it has additional methods that make it easy to create publication-ready figures in R. One of these methods is `stat_compare_means()`.

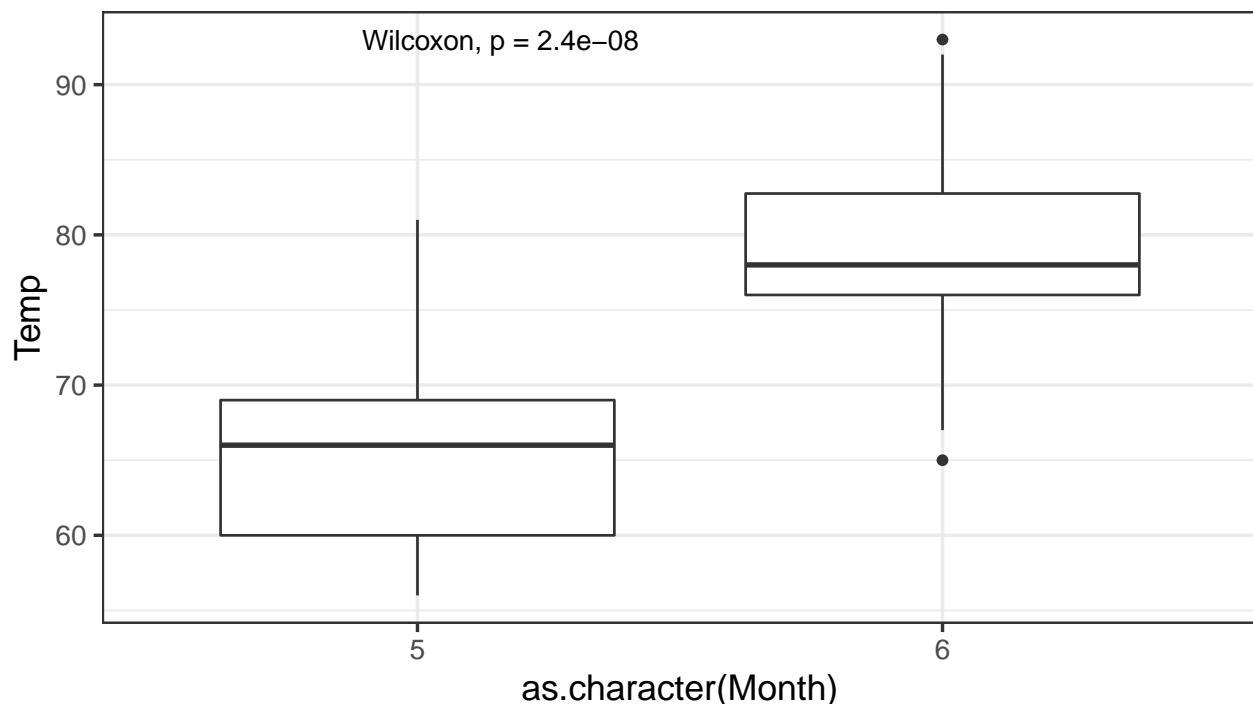
```
ggplot(airquality[airquality$Month %in% c(5, 6), ], aes(x = Month, y = Temp)) + geom_boxplot() +  
  stat_compare_means() + theme_bw(base_size = 14)
```

You have probably run into an error message as you run the code above.

Warning message: Continuous x aesthetic – did you forget aes(group=...)? .

This is because the categories we are passing to perform the paired test for significance are numeric (hence 'continuous'). We can overcome this by treating the category column's values (`Month`) as strings.

```
ggplot(airquality[airquality$Month %in% c(5, 6), ], aes(x = as.character(Month),  
  y = Temp)) + geom_boxplot() + stat_compare_means() + theme_bw(base_size = 14)
```



- You can change the type of test that is performed. For example, try updating `stat_compare_means()` to `stat_compare_means(method="t.test")`

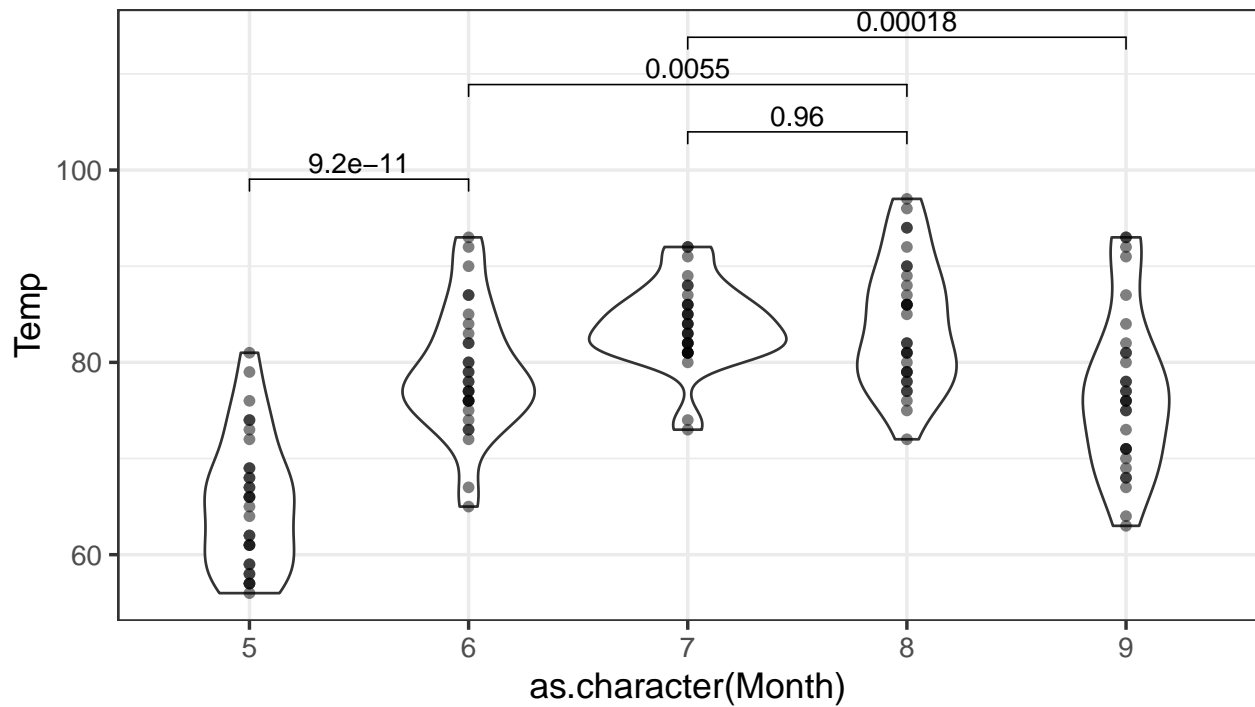
We can visualize the spread of data in the different categories using other geometric objects. A **violin plot** is an extension of a box-plot that shows the kernel density distributions of the data points, in addition to the median value and spread.

```
ggplot(airquality[airquality$Month %in% c(5, 6), ], aes(x = as.character(Month),
  y = Temp)) + geom_violin() + stat_compare_means() + theme_bw(base_size = 14)
```



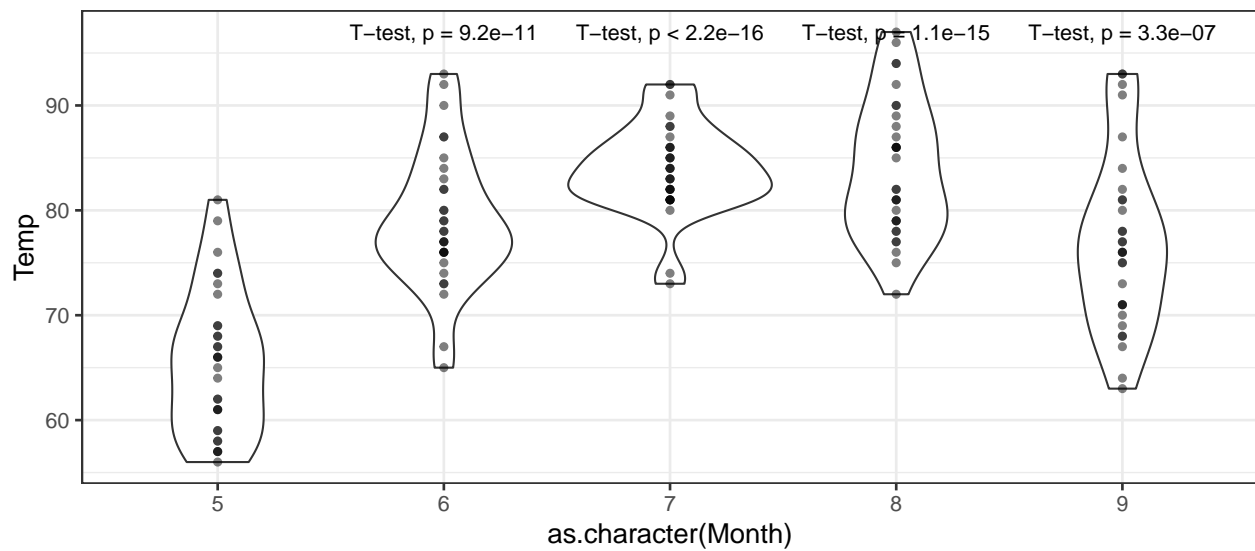
We can also extend the comparison to more than two groups. This, however, requires a bit of work. We first need to define the various pairwise comparisons we wish to perform. Subsequently we pass this list of comparisons to `stat_compare_means`.

```
my_comparisons <- list(c("5", "6"), c("7", "8"), c("6", "8"), c("7", "9"))
## Plot p-values for specified comparisons
ggplot(airquality, aes(x = as.character(Month), y = Temp)) + geom_violin() + geom_point(alpha = 0.5) +
  stat_compare_means(comparisons = my_comparisons, method = "t.test") + theme_bw(base_size = 14)
```



You can calculate the significance of difference in means between all groups relative to a reference, like so:

```
ggplot(airquality, aes(x = as.character(Month), y = Temp)) + geom_violin() + geom_point(alpha = 0.5) +
  stat_compare_means(method = "t.test", ref.group = "5") + theme_bw(base_size = 14)
```

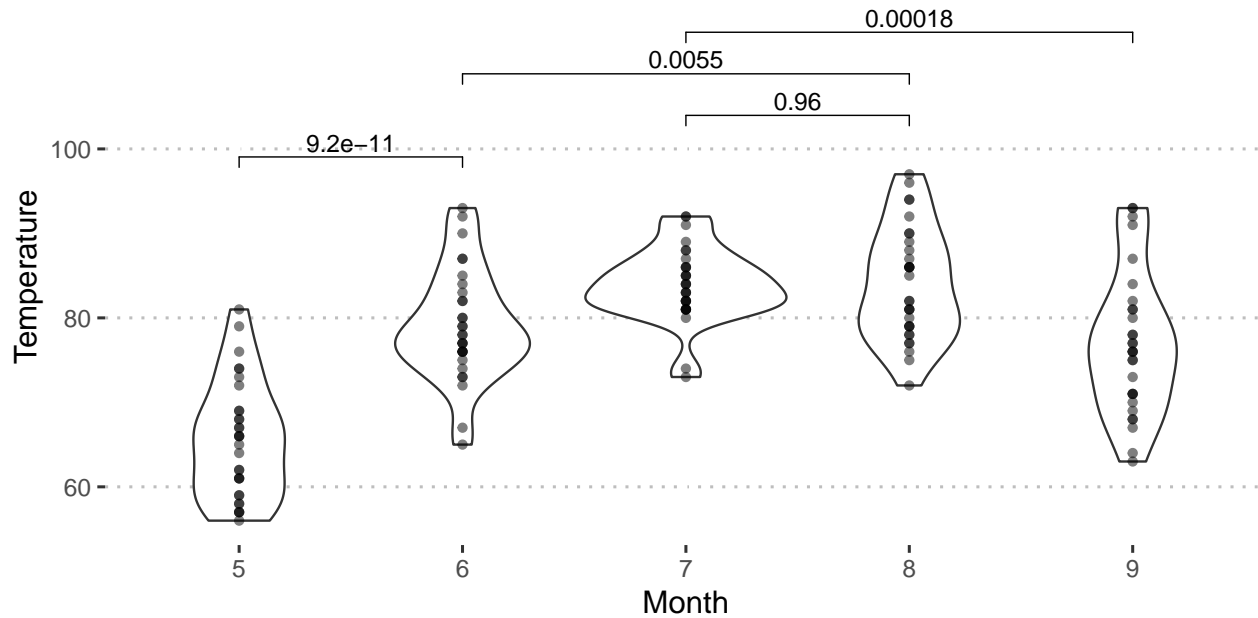


ggpubr magic

ggpubr's methods `theme_pubclean` and `theme_pubr` shift the focus of your plot to your data.

```
my_comparisons <- list(c("5", "6"), c("7", "8"), c("6", "8"), c("7", "9"))
```

```
ggplot(airquality, aes(x = as.character(Month), y = Temp)) + geom_violin() + geom_point(alpha = 0.5) +  
  stat_compare_means(comparisons = my_comparisons, method = "t.test") + labs(x = "Month",  
  y = "Temperature") + theme_pubclean(base_size = 14)
```



Additional Resources

Understanding reshape2, wide and long formats

Outlier detection with R

Detailed lecture on data cleanup with R

Using ggpubr to calculate significance

Take-aways

1. Basic smell-tests on your data
2. Removing cases with missing data
3. Identifying outliers
4. Descriptive statistics
5. Using ggpubr to create publication-ready figures

Extra

Pairs plots for expression data

Given a few samples (observations) with a large number of genes (variables), we can quickly evaluate if certain samples are outliers, simply by comparing the pair-wise scatterplots for all the samples

```
## We can also plot pairwise scatterplots
brca_df = data_merged[data_merged$cl_disease_detail %in% c("normal", "breast medullary carcinoma"),
  ]
## Reverse the melt step
brca_df_recast = dcast(brca_df[, c("Sample", "Gene", "Expression")], Sample ~ Gene)
rownames(brca_df_recast) = brca_df_recast$Sample
brca_df_recast$Sample <- NULL
## Remove the outlier gene
brca_df_recast = brca_df_recast[, !(colnames(brca_df_recast) %in% c("TSPAN6"))]
pairs(t(brca_df_recast), panel = function(...) smoothScatter(..., add = TRUE))
```

Filtering genes by average value or standard deviation

We firstly identify genes that vary within disease types. We will compare breast adenocarcinomas and breast ductal carcinomas.

```
## Select the samples from the metadata dataframe
samples_brca = metadata_df[metadata_df$cl_disease_detail %in% c("breast adenocarcinoma",
  "breast ductal carcinoma"), ]
## Filter our dataframe based on this list
brca_cohorts_df = data_df_clean2[rownames(data_df_clean2) %in% samples_brca$cl_id,
  ]
### Compare this dataframe to what you get with the following command
brca_cohorts_testdf = data_df_clean2[samples_brca$cl_id, ]
```

We will do some filtering to identify the most variable genes. Bioconductor's package `genefilter` also has some of these pre-implemented.

```
# Calculate mean of each gene
avg_genes = sapply(brca_cohorts_df, mean)
# Calculate standard deviation of each gene
sd_genes = sapply(brca_cohorts_df, sd)
# Filter dataframe based on an SD threshold of your choosing
brca_cohorts_filt = brca_cohorts_df[, names(sd_genes[sd_genes == max(sd_genes)]),
  drop = FALSE]
brca_cohorts_filt$cl_id = rownames(brca_cohorts_filt)
brca_cohorts_filt = merge(brca_cohorts_filt, metadata_df)

ggplot(brca_cohorts_filt, aes(x = cl_disease_detail, y = TFF1)) + geom_boxplot() +
  theme_bw(base_size = 16) + stat_compare_means(method = "t.test")
```

