



Statistical tools for high-throughput data analysis

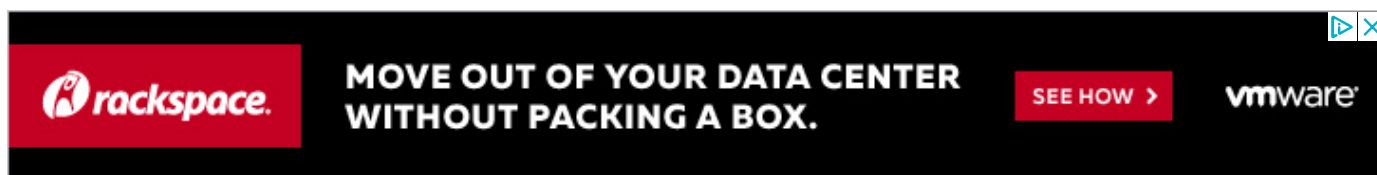
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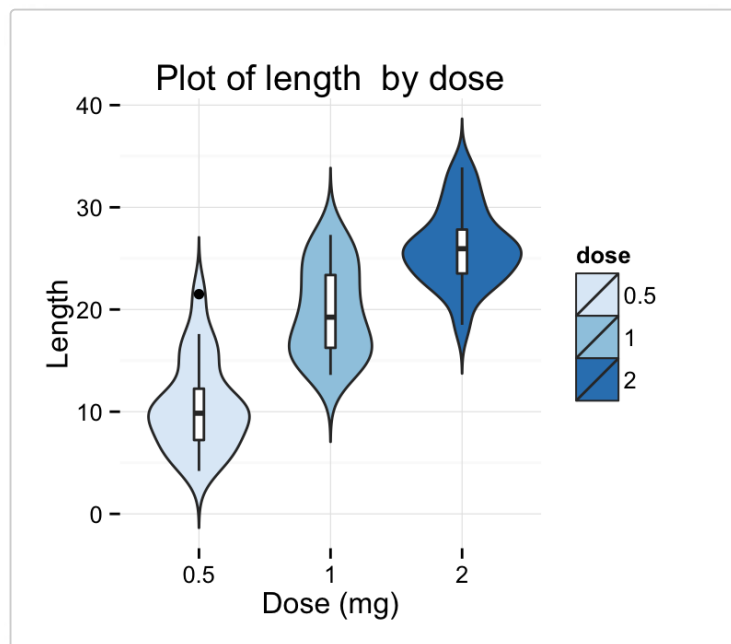
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This **R tutorial** describes how to create a **violin plot** using **R software** and **ggplot2** package.

violin plots are similar to **box plots**, except that they also show the kernel probability density of the data at different values. Typically, violin plots will include a marker for the median of the data and a box indicating the interquartile range, as in standard box plots.

The function **geom_violin()** is used to produce a violin plot.



Prepare the data

ToothGrowth data sets are used :

```
# Convert the variable dose from a numeric to a factor variable
ToothGrowth$dose <- as.factor(ToothGrowth$dose)
head(ToothGrowth)
```

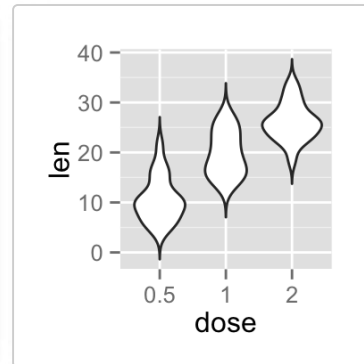
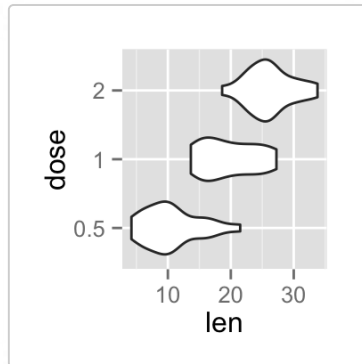
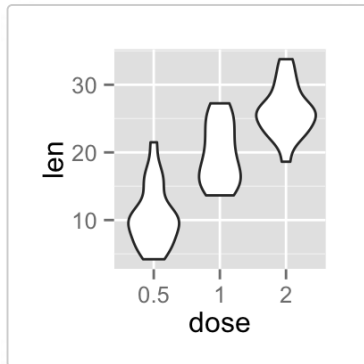
```
##      len supp dose
## 1  4.2   VC  0.5
## 2 11.5   VC  0.5
## 3  7.3   VC  0.5
## 4  5.8   VC  0.5
## 5  6.4   VC  0.5
## 6 10.0   VC  0.5
```



Make sure that the variable *dose* is converted as a factor variable using the above R script.

Basic violin plots

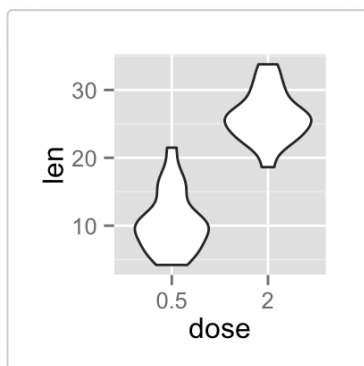
```
library(ggplot2)
# Basic violin plot
p <- ggplot(ToothGrowth, aes(x=dose, y=len)) +
  geom_violin()
p
# Rotate the violin plot
p + coord_flip()
# Set trim argument to FALSE
ggplot(ToothGrowth, aes(x=dose, y=len)) +
  geom_violin(trim=FALSE)
```



Note that by default *trim* = *TRUE*. In this case, the tails of the violins are trimmed. If *FALSE*, don't trim the tails

Choose which items to display :

```
p + scale_x_discrete(limits=c("0.5", "2"))
```

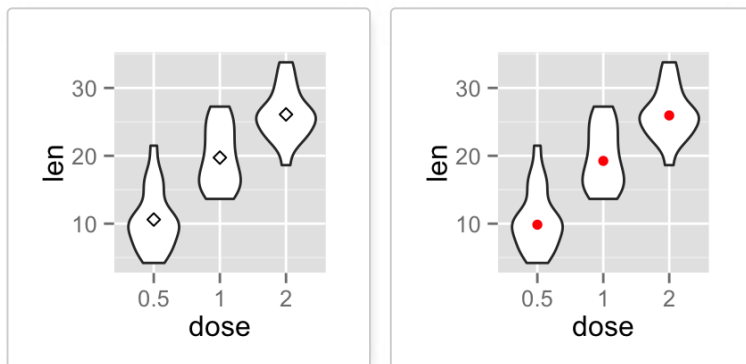


Add summary statistics on a violin plot

The function **stat_summary()** can be used to add mean/median points and more on a violin plot.

Add mean and median points

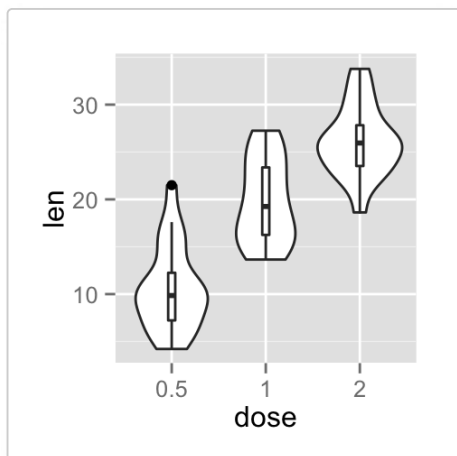
```
# violin plot with mean points
p + stat_summary(fun.y=mean, geom="point", shape=23, size=2)
# violin plot with median points
p + stat_summary(fun.y=median, geom="point", size=2, color="red")
```



Add median and quartile

A solution is to use the function **geom_boxplot** :

```
p + geom_boxplot(width=0.1)
```



Add mean and standard deviation

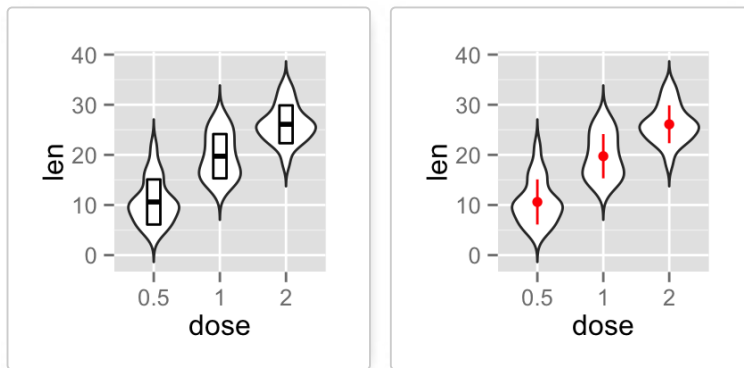
The function **mean_sdl** is used. *mean_sdl* computes the *mean* plus or minus a *constant* times the *standard deviation*.

In the R code below, the constant is specified using the argument *mult* (mult = 1). By default mult = 2.

The mean +/- SD can be added as a *crossbar* or a *pointrange* :

```
p <- ggplot(ToothGrowth, aes(x=dose, y=len)) +
  geom_violin(trim=FALSE)
p + stat_summary(fun.data="mean_sdl", mult=1,
  geom="crossbar", width=0.2 )
```

```
p + stat_summary(fun.data=mean_sd1, mult=1,
  geom="pointrange", color="red")
```

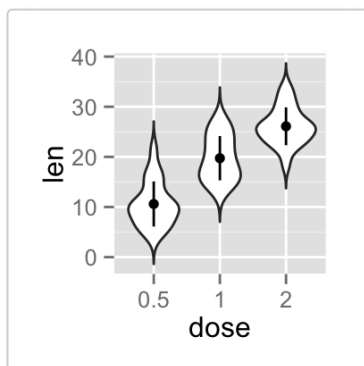


Note that, you can also define a custom function to produce summary statistics as follow :

```
# Function to produce summary statistics (mean and +/- sd)
data_summary <- function(x) {
  m <- mean(x)
  ymin <- m-sd(x)
  ymax <- m+sd(x)
  return(c(y=m,ymin=ymin,ymax=ymax))
}
```

Use a custom summary function :

```
p + stat_summary(fun.data=data_summary)
```

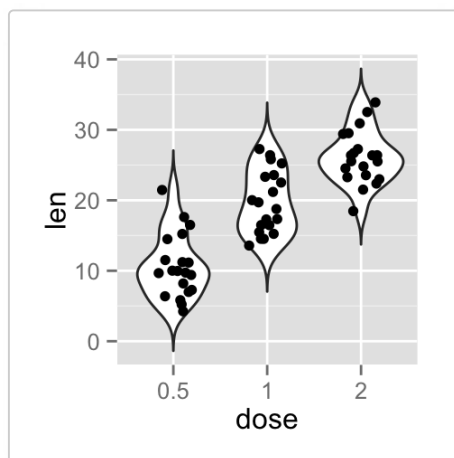


Violin plot with dots

Dots (or points) can be added to a violin plot using the functions **geom_dotplot()** or **geom_jitter()** :

```
# violin plot with dot plot
p + geom_dotplot(binaxis='y', stackdir='center', dotsize=1)
```

```
# violin plot with jittered points
# 0.2 : degree of jitter in x direction
p + geom_jitter(shape=16, position=position_jitter(0.2))
```

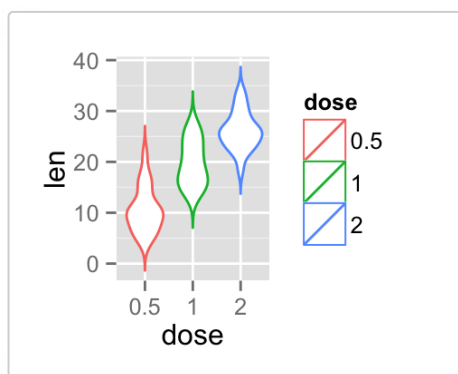


Change violin plot colors by groups

Change violin plot line colors

Violin plot line colors can be automatically controlled by the levels of *dose* :

```
# Change violin plot line colors by groups
p<-ggplot(ToothGrowth, aes(x=dose, y=len, color=dose)) +
  geom_violin(trim=FALSE)
p
```

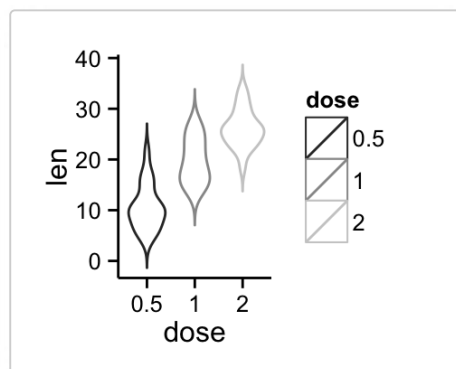
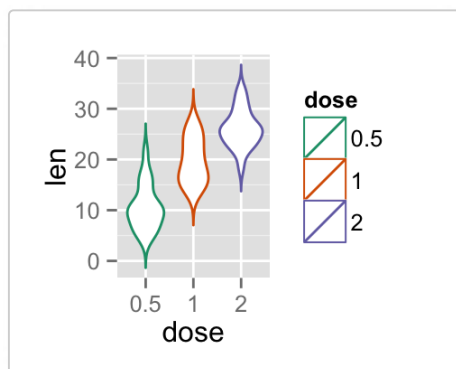
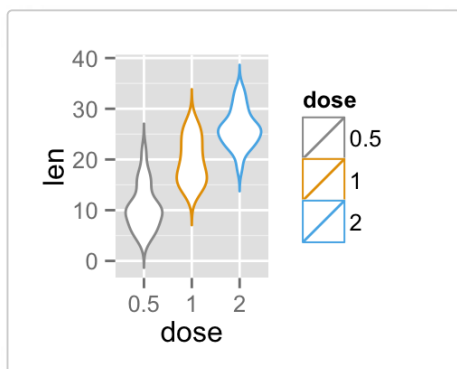


It is also possible to *change manually violin plot line colors* using the functions :

- `scale_color_manual()` : to use custom colors
- `scale_color_brewer()` : to use color palettes from *RColorBrewer* package
- `scale_color_grey()` : to use grey color palettes

```
# Use custom color palettes
p+scale_color_manual(values=c("#999999", "#E69F00", "#56B4E9"))
```

```
# Use brewer color palettes
p+scale_color_brewer(palette="Dark2")
# Use grey scale
p + scale_color_grey() + theme_classic()
```

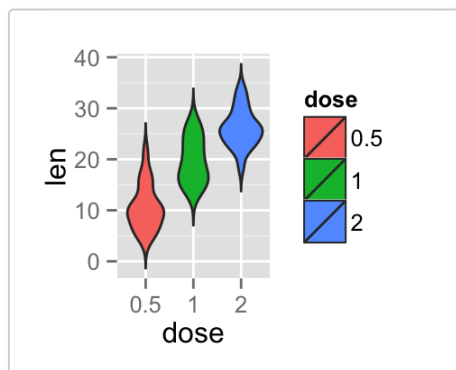
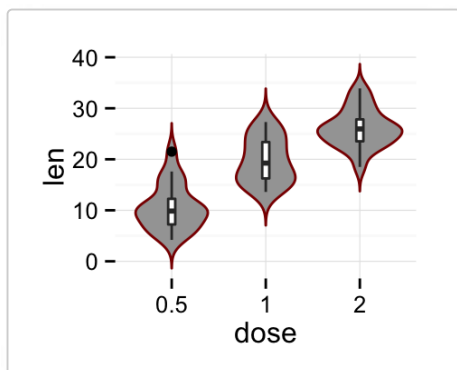


Read more on ggplot2 colors here : [ggplot2 colors](#)

Change violin plot fill colors

In the R code below, the fill colors of the violin plot are automatically controlled by the levels of *dose* :

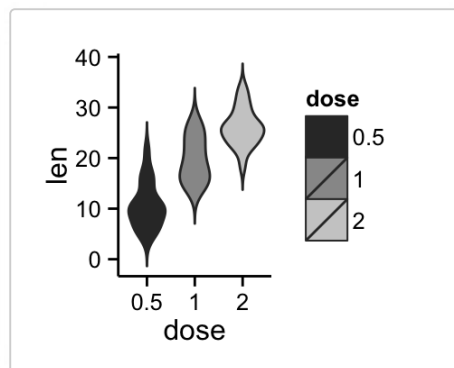
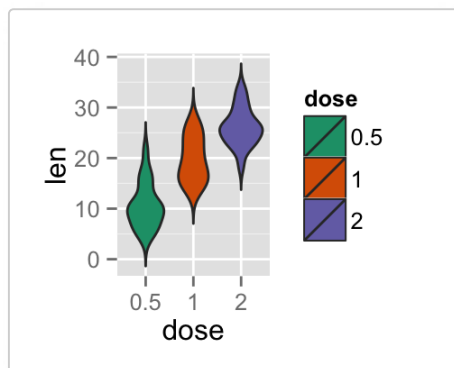
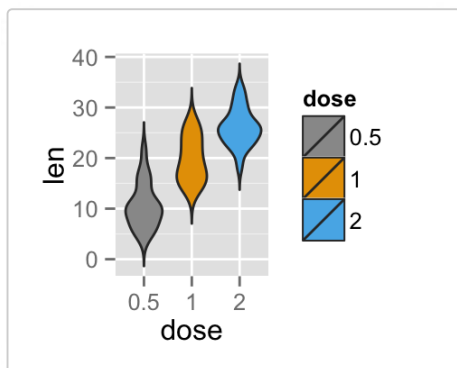
```
# Use single color
ggplot(ToothGrowth, aes(x=dose, y=len)) +
  geom_violin(trim=FALSE, fill='#A4A4A4', color="darkred")+
  geom_boxplot(width=0.1) + theme_minimal()
# Change violin plot colors by groups
p<-ggplot(ToothGrowth, aes(x=dose, y=len, fill=dose)) +
  geom_violin(trim=FALSE)
p
```



It is also possible to *change manually violin plot colors* using the functions :

- `scale_fill_manual()` : to use custom colors
- `scale_fill_brewer()` : to use color palettes from *RColorBrewer* package
- `scale_fill_grey()` : to use grey color palettes

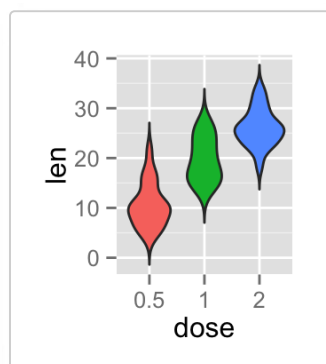
```
# Use custom color palettes
p+scale_fill_manual(values=c("#999999", "#E69F00", "#56B4E9"))
# Use brewer color palettes
p+scale_fill_brewer(palette="Dark2")
# Use grey scale
p + scale_fill_grey() + theme_classic()
```



Read more on ggplot2 colors here : [ggplot2 colors](#)

Change the legend position

```
p + theme(legend.position="top")
p + theme(legend.position="bottom")
p + theme(legend.position="none") # Remove legend
```



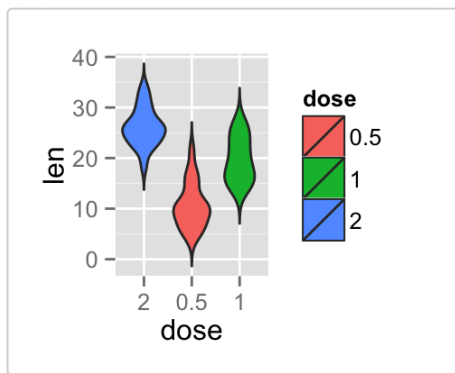
✓ The allowed values for the arguments **legend.position** are : "left", "top", "right", "bottom".

Read more on ggplot legends : [ggplot2 legend](#)

Change the order of items in the legend

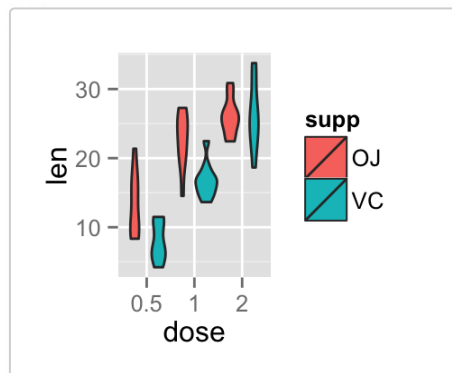
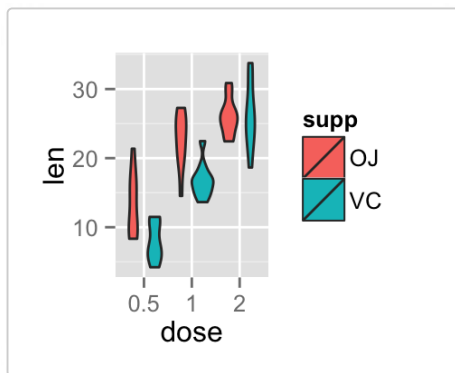
The function **scale_x_discrete** can be used to change the order of items to "2", "0.5", "1" :

```
p + scale_x_discrete(limits=c("2", "0.5", "1"))
```

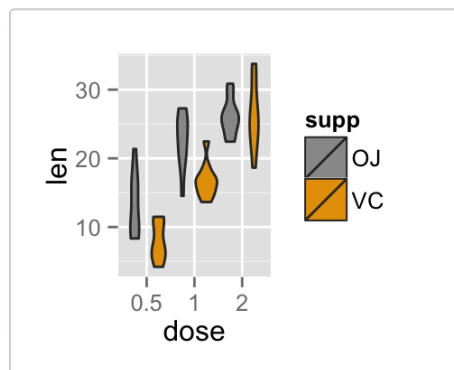
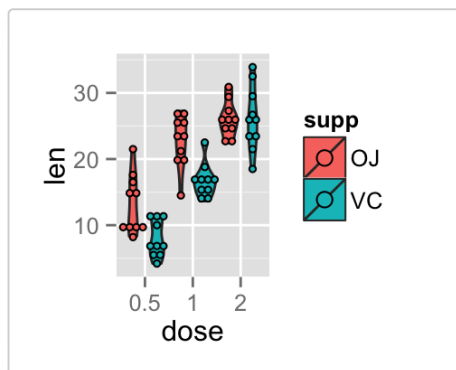
Violin plot with multiple groups

```
# Change violin plot colors by groups
ggplot(ToothGrowth, aes(x=dose, y=len, fill=supp)) +
  geom_violin()
# Change the position
p<-ggplot(ToothGrowth, aes(x=dose, y=len, fill=supp)) +
  geom_violin(position=position_dodge(1))
p
```



Change violin plot colors and add dots :

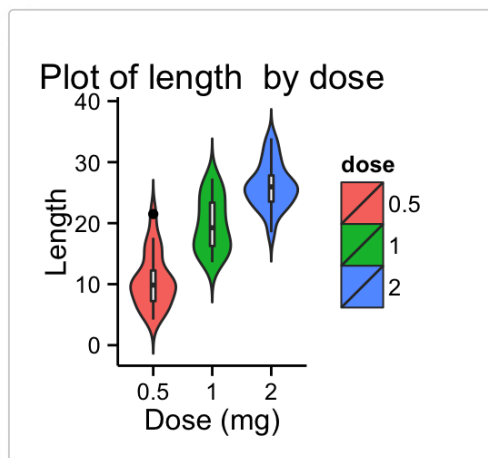
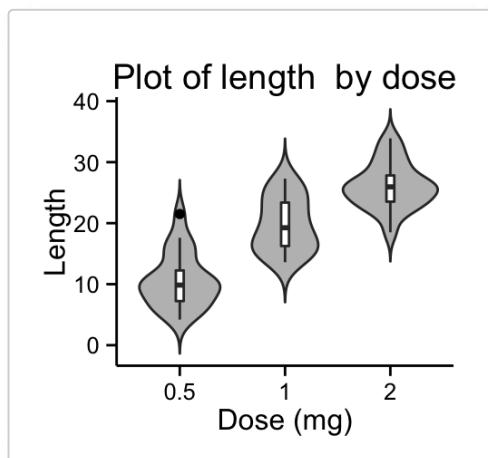
```
# Add dots
p + geom_dotplot(binaxis='y', stackdir='center',
  position=position_dodge(1))
# Change colors
p+scale_fill_manual(values=c("#999999", "#E69F00", "#56B4E9"))
```



Customized violin plots

```
# Basic violin plot
ggplot(ToothGrowth, aes(x=dose, y=len)) +
  geom_violin(trim=FALSE, fill="gray")+
  labs(title="Plot of length by dose", x="Dose (mg)", y = "Length")+
  geom_boxplot(width=0.1)+
  theme_classic()

# Change color by groups
dp <- ggplot(ToothGrowth, aes(x=dose, y=len, fill=dose)) +
  geom_violin(trim=FALSE)+
  geom_boxplot(width=0.1, fill="white")+
  labs(title="Plot of length by dose", x="Dose (mg)", y = "Length")
dp + theme_classic()
```



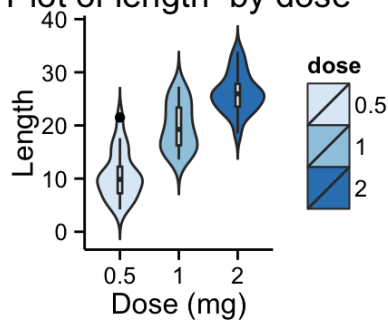
Change fill colors manually :

```
# Continuous colors
dp + scale_fill_brewer(palette="Blues") + theme_classic()

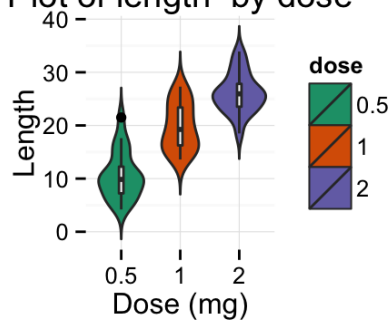
# Discrete colors
dp + scale_fill_brewer(palette="Dark2") + theme_minimal()

# Gradient colors
dp + scale_fill_brewer(palette="RdBu") + theme_minimal()
```

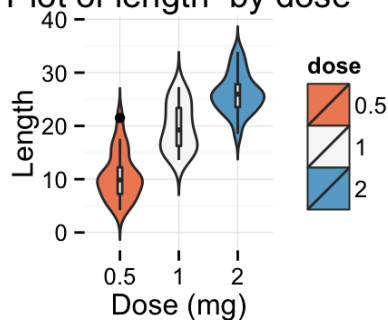
Plot of length by dose



Plot of length by dose



Plot of length by dose



Read more on ggplot2 colors here : [ggplot2 colors](#)

Infos



This analysis has been performed using **R software** (ver. 3.1.2) and **ggplot2** (ver. 1.0.0)



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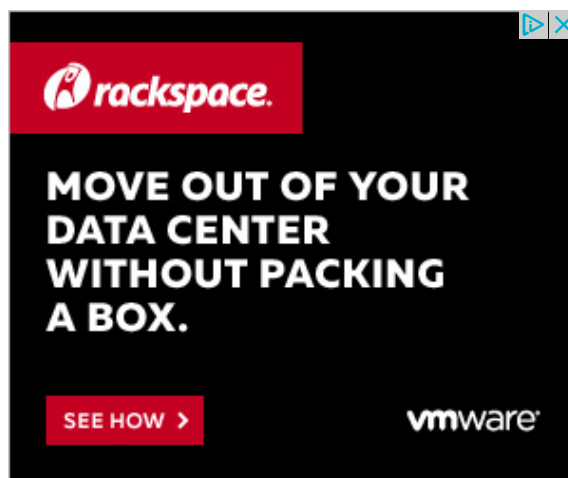
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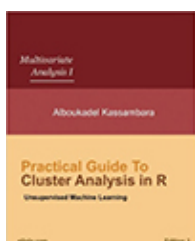
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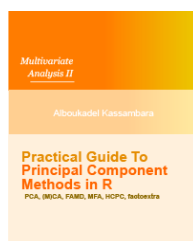
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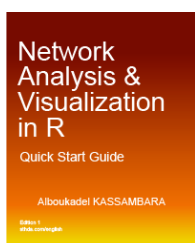
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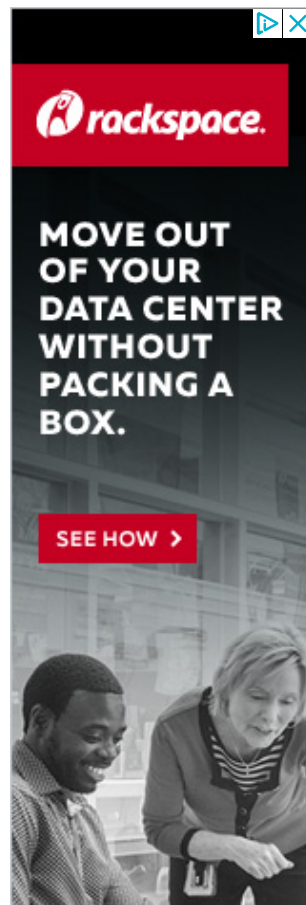
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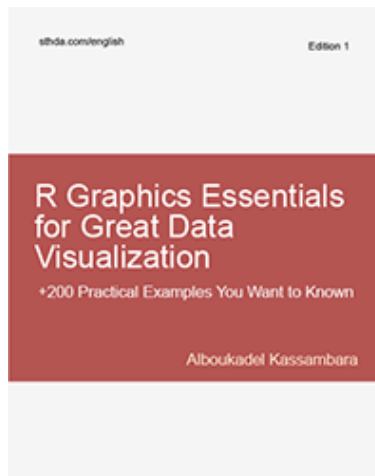
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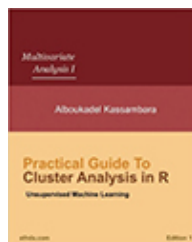
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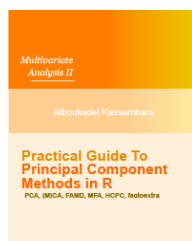
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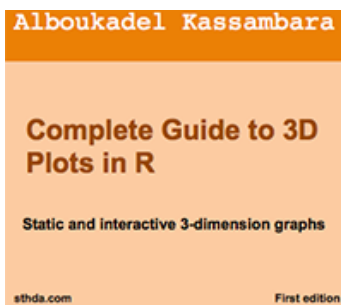
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