Swiss Institute of Bioinformatics

First Steps with R in Life Sciences: Statistics

With slides from: Diana Marek, Geoffrey Fucile, Alex Smith, Linda Dib, Leonore Wigger, Wandrille Duchemin

elixir SWITZERLAND

Statistical hypothesis testing

Two **hypotheses in competition**:

- Ho: the **NULL hypothesis** (usually the most conservative – e.g., "no difference")
- H1: the **alternative hypothesis** (usually the one we are actually interested in)

Example:

Ho: « There is no difference in weight between two given strains of mice »

H1: « The average weight in KO mice is different from that in WT mice »

Statistical test:

- Calculate test statistic,
- Calculate associated p-value,
- Check if p-value is small enough to reject Ho, according to pre-defined significance level.

t-test

Goal:

- Compare a continuous measure between two groups
- Is the difference between the two group means statistically significant?

Assumptions:

- Observations are independent
- The two groups follow a normal distribution
- ~~(Same variance in each group)~~

R uses Welch's t-test, which does not assume equal variance

Example data set: iris

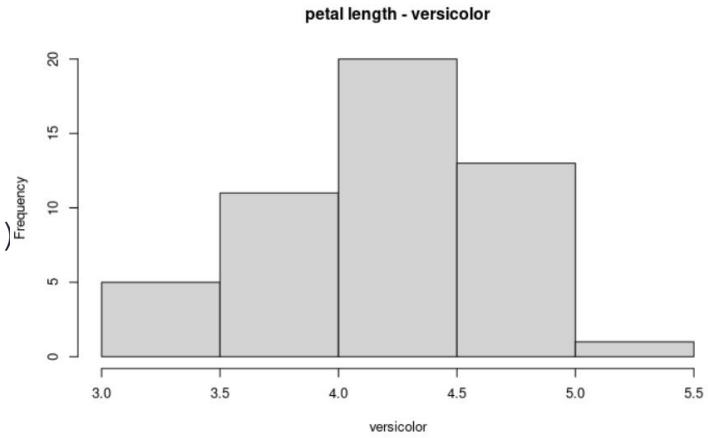
Let's compare *Iris virginica* and *Iris versicolor* petal lengths.

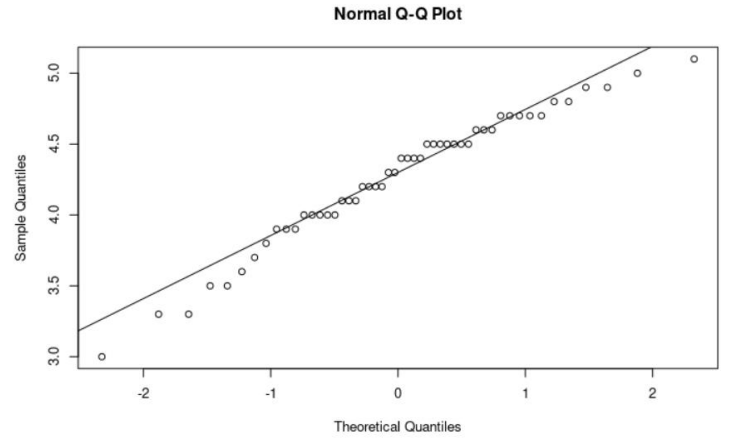
```
data(iris)
# we limit the data to 2 species
iris_f = iris[ iris$Species %in% c('versicolor','virginica') , ]
iris_f$Species = factor(iris_f$Species)

tapply( iris_f$Petal.Length, iris_f$Species, mean )
versicolor  virginica
     4.260      5.552

plen_versicolor = iris_f$Petal.Length[iris_f$Species=='versicolor']
plen_virginica  = iris_f$Petal.Length[iris_f$Species=='virginica']
```

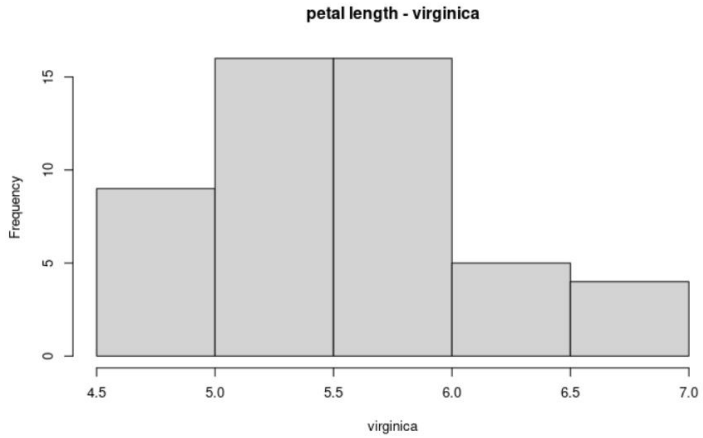
Check normality of the data with plots

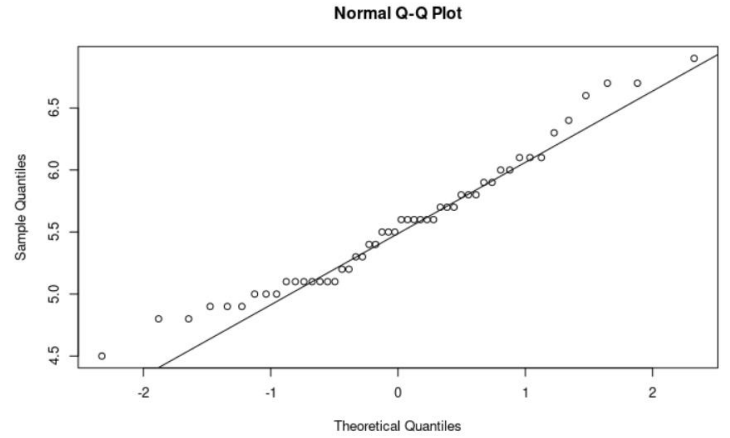
We use histograms and QQplot to visually assess normality

```
par(mfrow=c(2,2))
hist(plen_versicolor,
     xlab="versicolor",
     main="petal length - versicolor")
qqnorm(plen_versicolor)
qqline(plen_versicolor)

hist(plen_virginica,
     xlab="virginica",
     main="petal length - virginica")
qqnorm(plen_virginica)
qqline(plen_virginica)
dev.off()
```







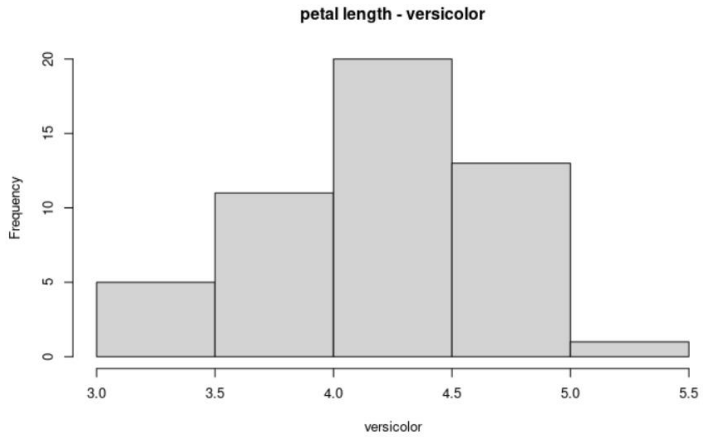


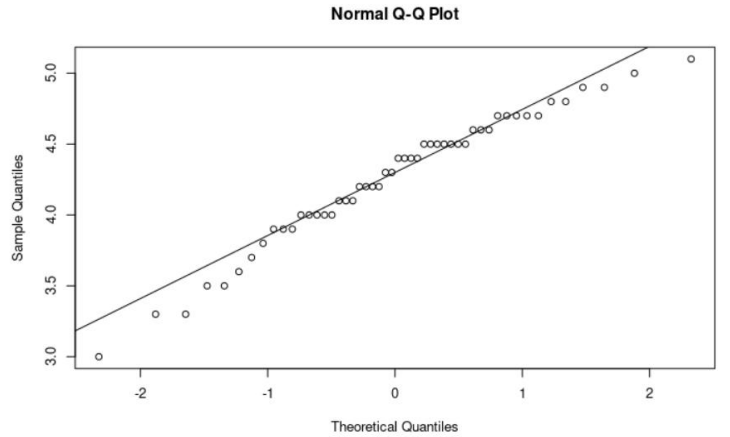
Check normality of the data with tests

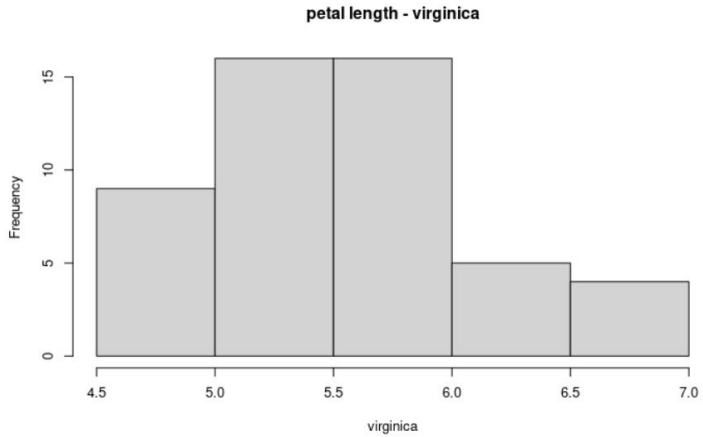
We use the Shapiro-Wilks test, it is not ideal as the null hypothesis is normality (what we would like). So, accepting normality has no type II error control…

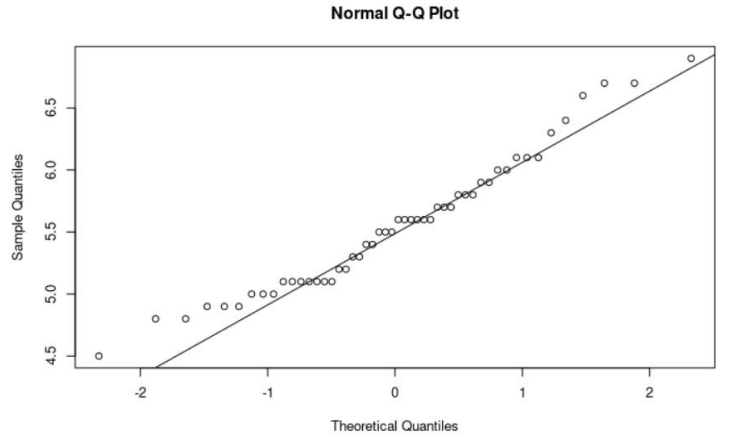
```
shapiro.test(plen_versicolor)
    Shapiro-Wilk normality test

data:  plen_versicolor
W = 0.966, p-value = 0.1585
```

```
shapiro.test(plen_virginica)
    Shapiro-Wilk normality test

data:  plen_virginica
W = 0.96219, p-value = 0.1098
```









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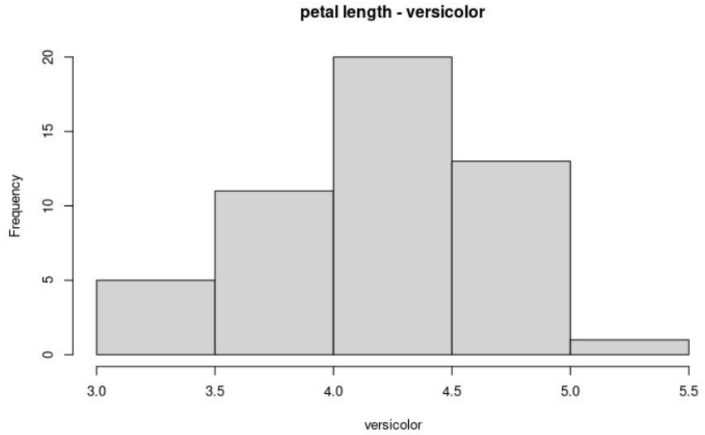
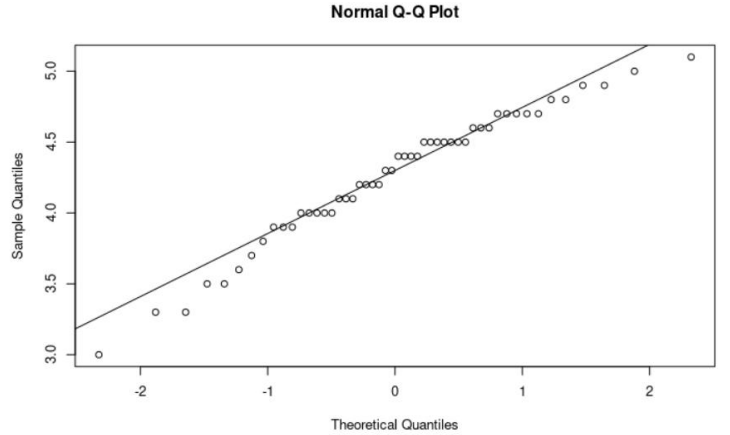
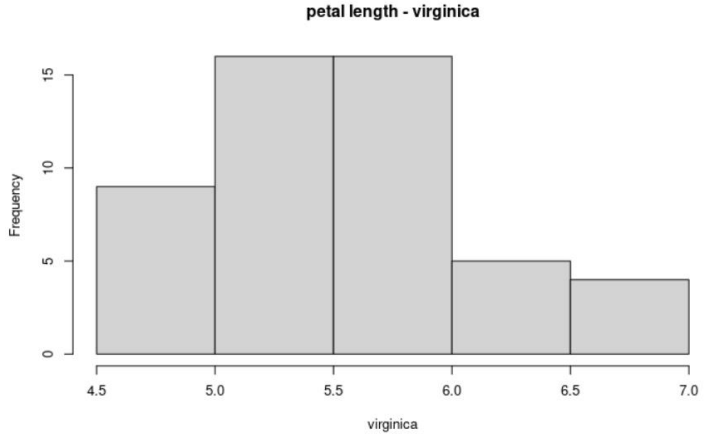
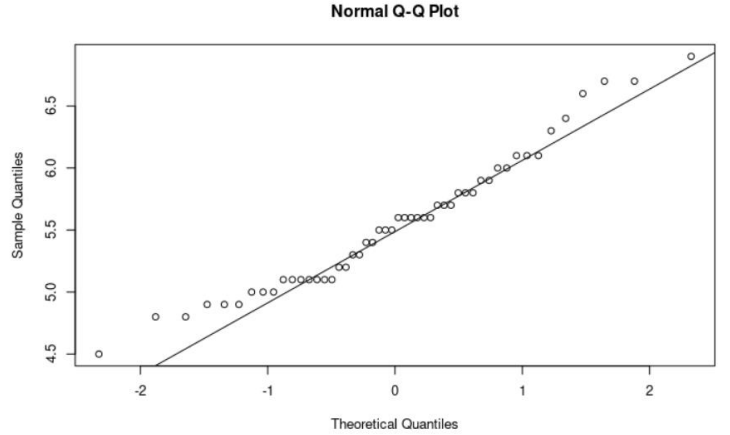




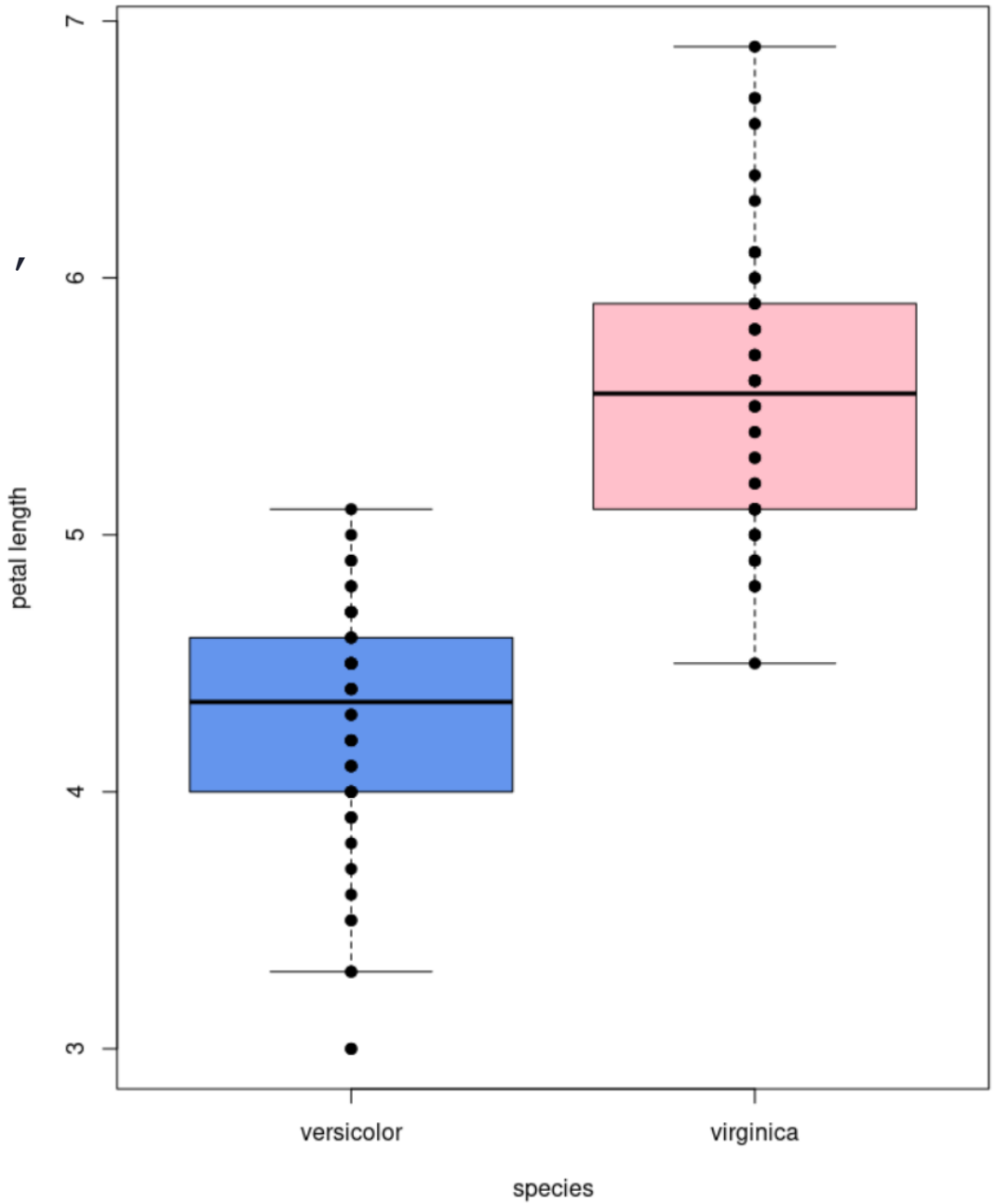




t-test is somewhat robust to non-normal data. No need to be too strict about normality requirement.

Visualize group differences

```
boxplot(Petal.Length ~ Species,
        data=iris_f,
        col=c("cornflowerblue", "pink"),
        ylab="petal length",
        xlab="species")
points( Petal.Length ~ Species,
        data=iris_f,
        col="black",
        pch = 19)
```



Function t.test()

```
t.test(plen_versicolor, plen_virginica)


t.test(Petal.Length ~ Species, data=iris_f) #equivalent to the above


Welch Two Sample t-test


data:  Petal.Length by Species
t = -12.604, df = 95.57, p-value < 2.2e-16
alternative hypothesis: true difference in means between group versicolor and group
virginica is not equal to 0
95 percent confidence interval:
-1.49549 -1.08851
sample estimates:
mean in group versicolor  mean in group virginica
                   4.260                    5.552
```

t.test object

- t.test() and other tests return a **list** that can be assigned to a variable.
- View the names of the list's slots using **names()**.
- Access the elements of a list using the **$** or the **[[ ]]** operators.

```
test_res = t.test(Petal.Length ~ Species, data=iris_f)

names(test_res)
[1] "statistic"   "parameter"   "p.value"     "conf.int"    "estimate"
[6] "null.value"  "stderr"      "alternative" "method"      "data.name"

test_res[['p.value']]
[1] 4.900288e-22
```

Paired data

When the measurements correspond to 2 observations on the same individuals, we need to use the **paired t-test**.

This tests focuses on the difference between the two observations and asks if the mean of this difference is different from 0.

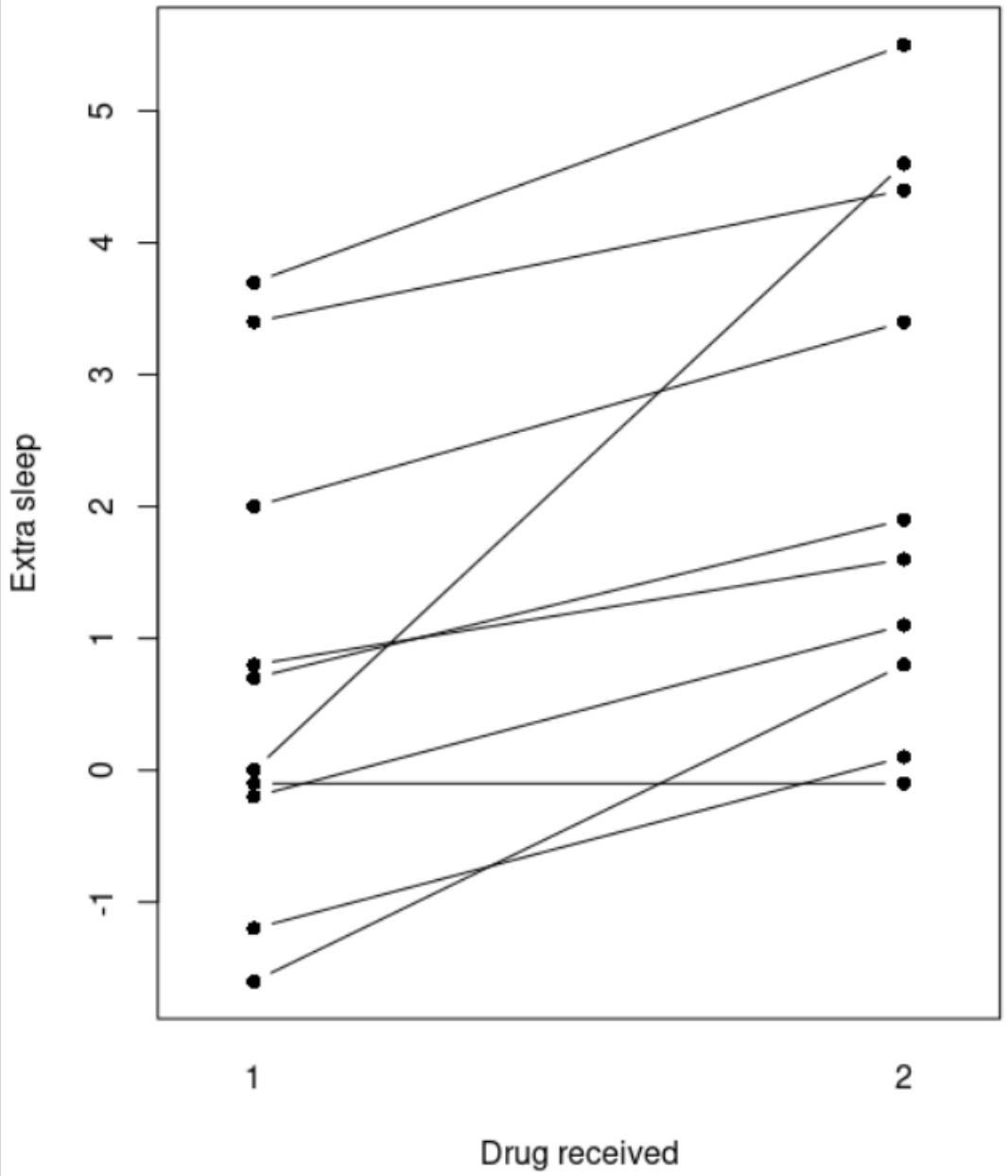
Assumption: the sample mean of the differences are normally distributed

Paired data representation

```
data(sleep)
head(sleep, n=3)
```

```
extra group ID
1   0.7     1  1
2  -1.6     1  2
3  -0.2     1  3
```

```
interaction.plot(response=sleep$extra,
                 x.factor=sleep$group,
                 trace.factor=sleep$ID,
                 legend=FALSE, type="b",
                 lty=1, pch=16,
                 xlab="Drug received",
                 ylab="Extra sleep")
```

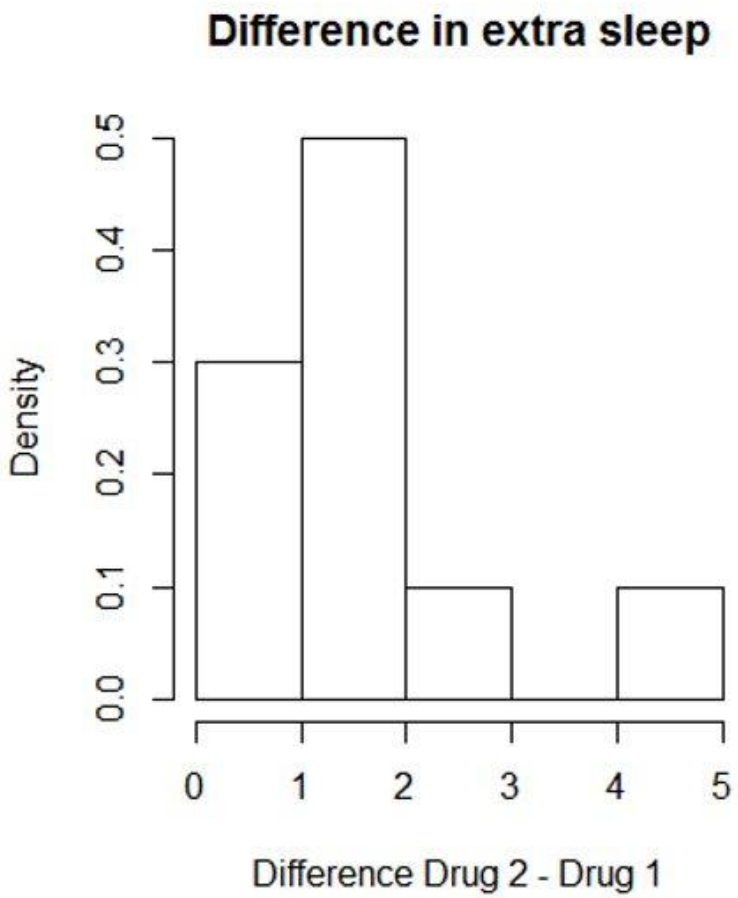


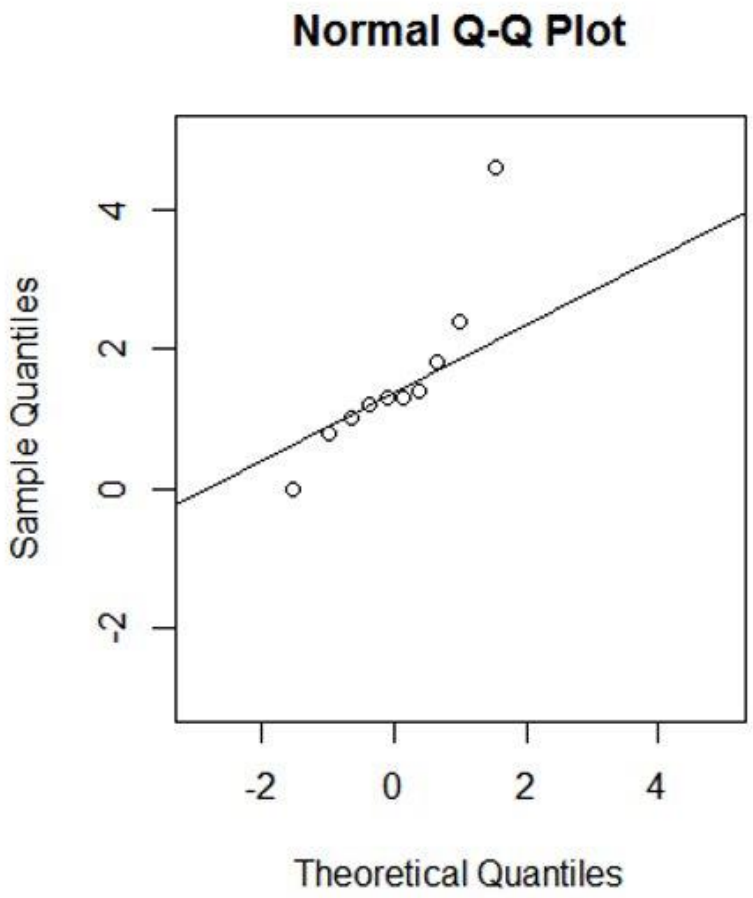
Check normality of the differences between pairs

```
difference = sleep$extra[sleep$group==2]-sleep$extra[sleep$group==1]

hist(difference, freq=FALSE, xlab="Difference Drug 2 - Drug 1",
                 main="Difference in extra sleep", col="white")

qqnorm(difference)
qqline(difference)
```





Paired t-test

```
# using a paired t-test:
t.test(sleep$extra[sleep$group==1],
       sleep$extra[sleep$group==2], paired=TRUE)
# paired values must be at the same position in the two vectors
# do not use formula notation (extra~sleep) for paired t-test
```

```
Paired t-test

data:  sleep$extra[sleep$group == 1] and sleep$extra[sleep$group == 2]
t = -4.0621, df = 9, p-value = 0.002833
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -2.4598858 -0.7001142
sample estimates:
mean of the differences
                  -1.58
```

Non-parametric alternatives to the t-test

When the data deviates strongly from normality, a **non-parametric test** can be used in place of a t-test.

Non-parametric tests **do not assume any particular distribution of the data**.

Instead of t-test (without pairing), use Mann-Whitney U test.

```
wilcox.test(sleep$extra[sleep$group==1],
            sleep$extra[sleep$group==2])
wilcox.test(extra~group, data=sleep) # equivalent
```

Instead of paired t-test, use Wilcoxon Signed Rank test.

```
wilcox.test(sleep$extra[sleep$group==1],
            sleep$extra[sleep$group==2], paired=TRUE)
```

These two tests have different names but are **both implemented in the R function wilcox.test**.

wilcox.test()

For the sleep data, a paired test is appropriate.

```
wilcox.test(sleep$extra[sleep$group==1],
            sleep$extra[sleep$group==2], paired=TRUE)
Wilcoxon signed rank test with continuity correction

data: sleep$extra[sleep$group==1] and sleep$extra[sleep$group==2]
V = 0, p-value = 0.009091
alternative hypothesis: true location shift is not equal to 0
```

The conclusion is the same as it was for the paired t-test.

- The p-value is a little higher wilcox.test: 0.009091 (t.test: 0.002833)

wilcox.test() - warning messages

wilcox.test() implements two ways to compute p-values: exact and by approximation

- The method can be selected with parameter **exact=TRUE** or **exact=FALSE**
- The default is "exact" if sample size < 50 *and* there are no ties in the data.
- Otherwise, it is by normal approximation.

```
Warning messages:
1: In wilcox.test.default(sleep$extra[sleep$group == 1], sleep$extra[sleep$group ==  :
  cannot compute exact p-value with ties
2: In wilcox.test.default(sleep$extra[sleep$group == 1], sleep$extra[sleep$group ==  :
  cannot compute exact p-value with zeroes
```

These **warnings don't mean that there is an error in the result**. An (approximated) p-value is still provided and can be reported.

Let's practice - 10

Come back to the mice data-set stored in the "mice_data" data frame.

1) Considering WT mice weight and KO mice weight separately, check the assumption of normality graphically.

2) Make an appropriate plot to visualize the mouse weights grouped by genotype.

3) Perform a test to see whether the mouse weight is different between the two genotypes.

*4) Repeat steps **1** to **3** for the diet variable.*

Bivariate linear correlation

Goal: Quantify the strength of a linear correlation between two continuous variables

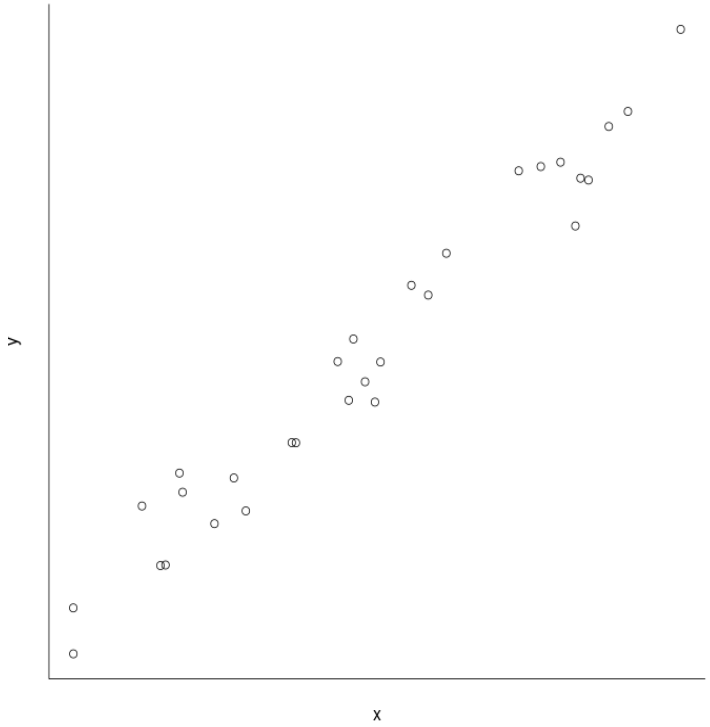
cor() computes a correlation between two variables.

- **Default: method="pearson"** (linear correlation)
- **Other options: method="spearman", method="kendall"** (rank-based correlations)

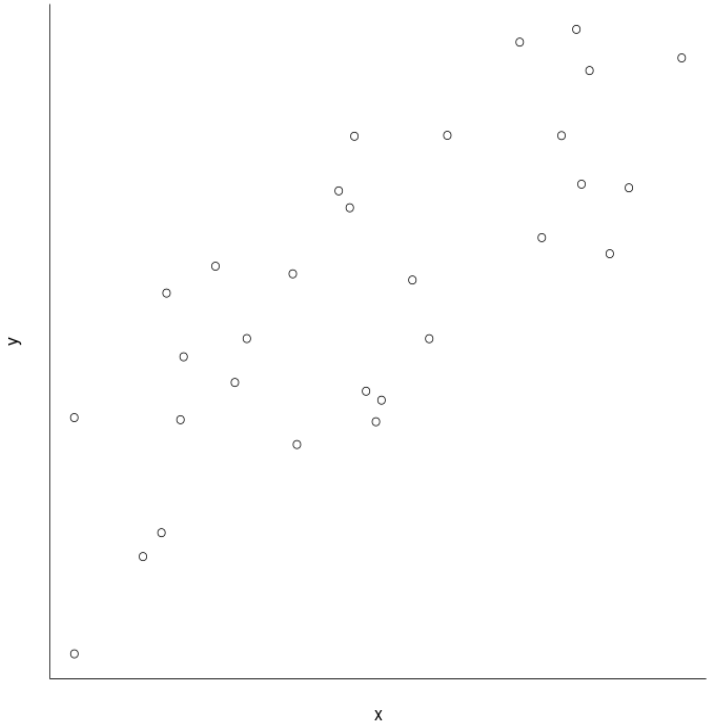
cor.test()

- computes a correlation and performs a corresponding statistical test
- for Pearson correlation: p-value from linear regression, same as lm()

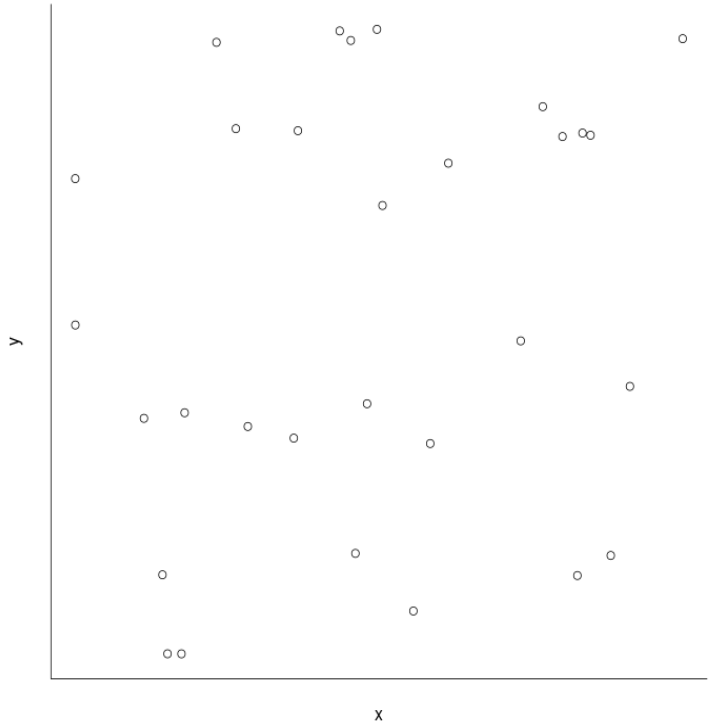
Visualization of linear correlation



Strong linear correlation

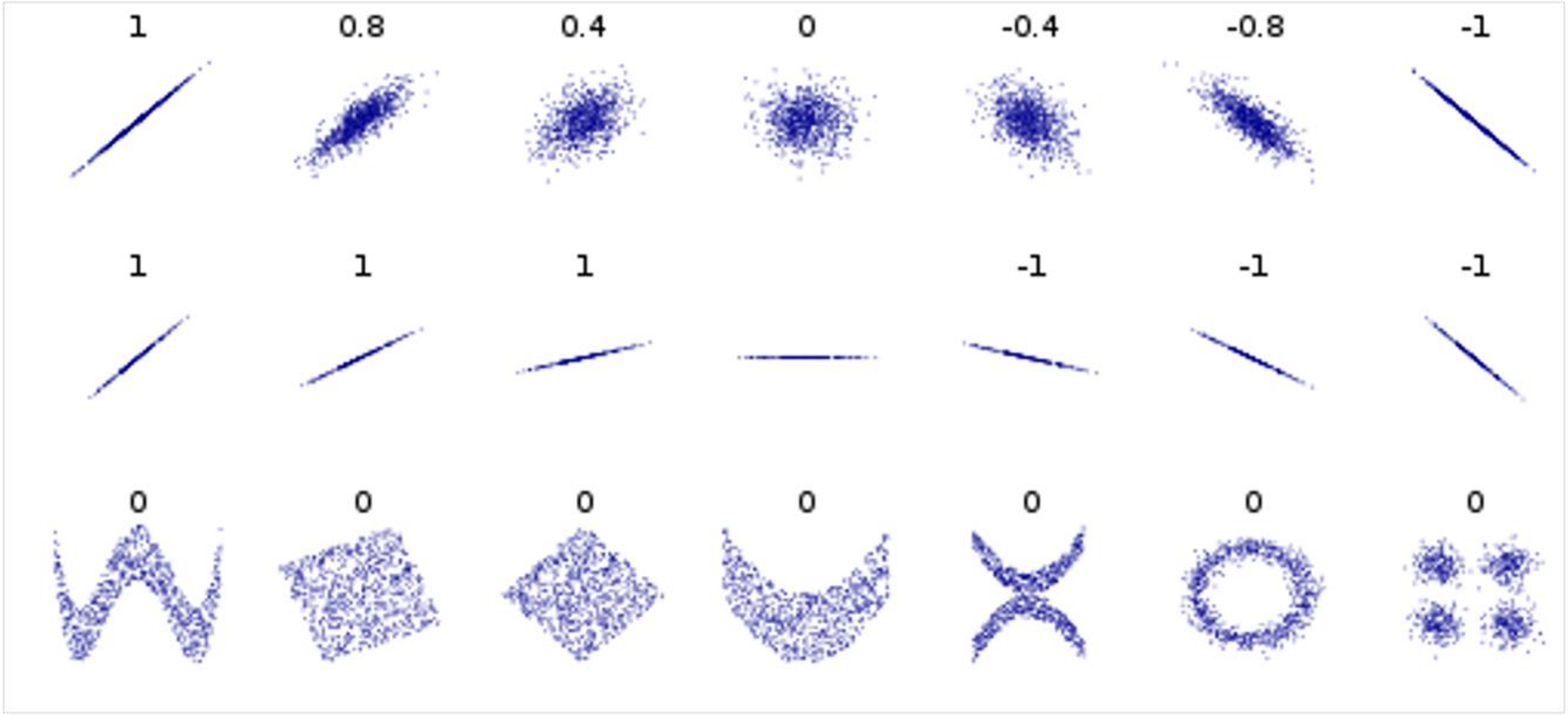


Medium-strong linear correlation



No correlation

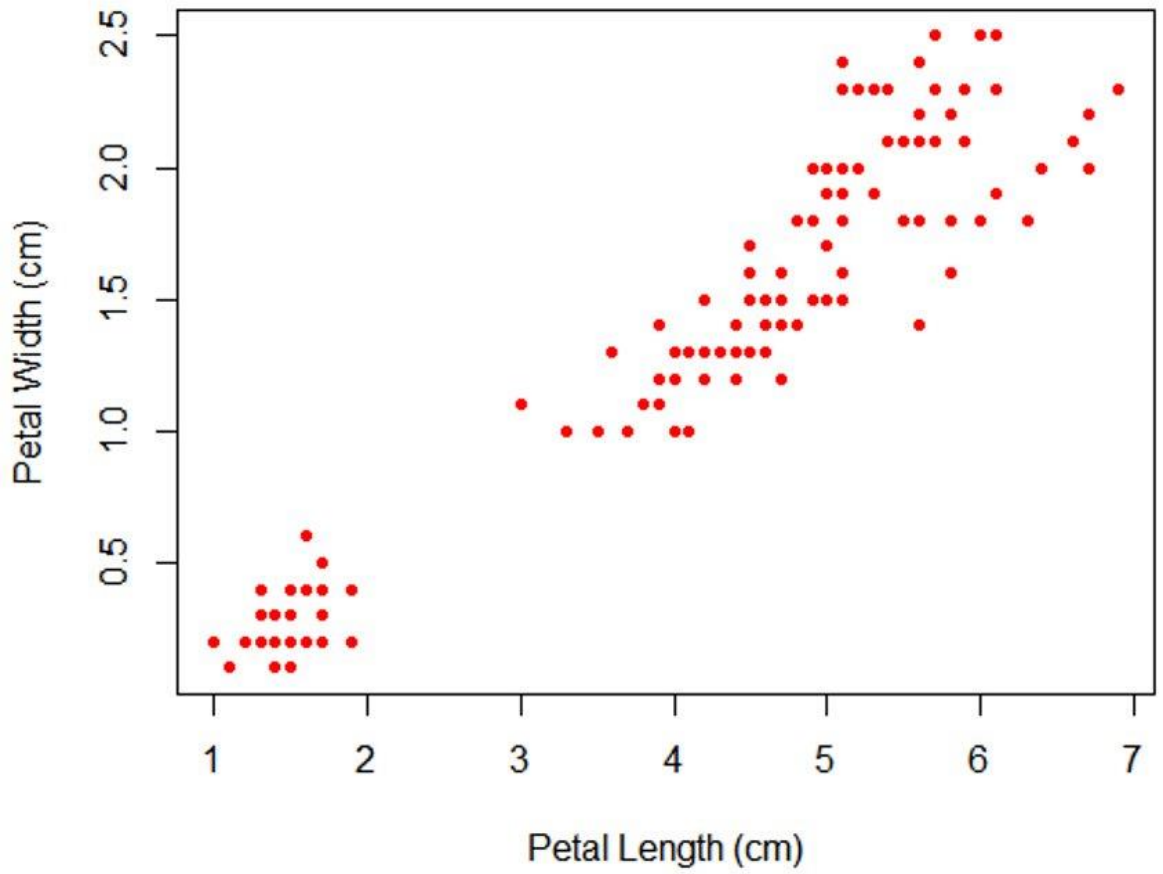
More vosialization of linear correlation



correlation reflects the noisiness and direction of a linear relationship (top row)
but not the slope of that relationship (middle)
nor many aspects of nonlinear relationships (bottom).

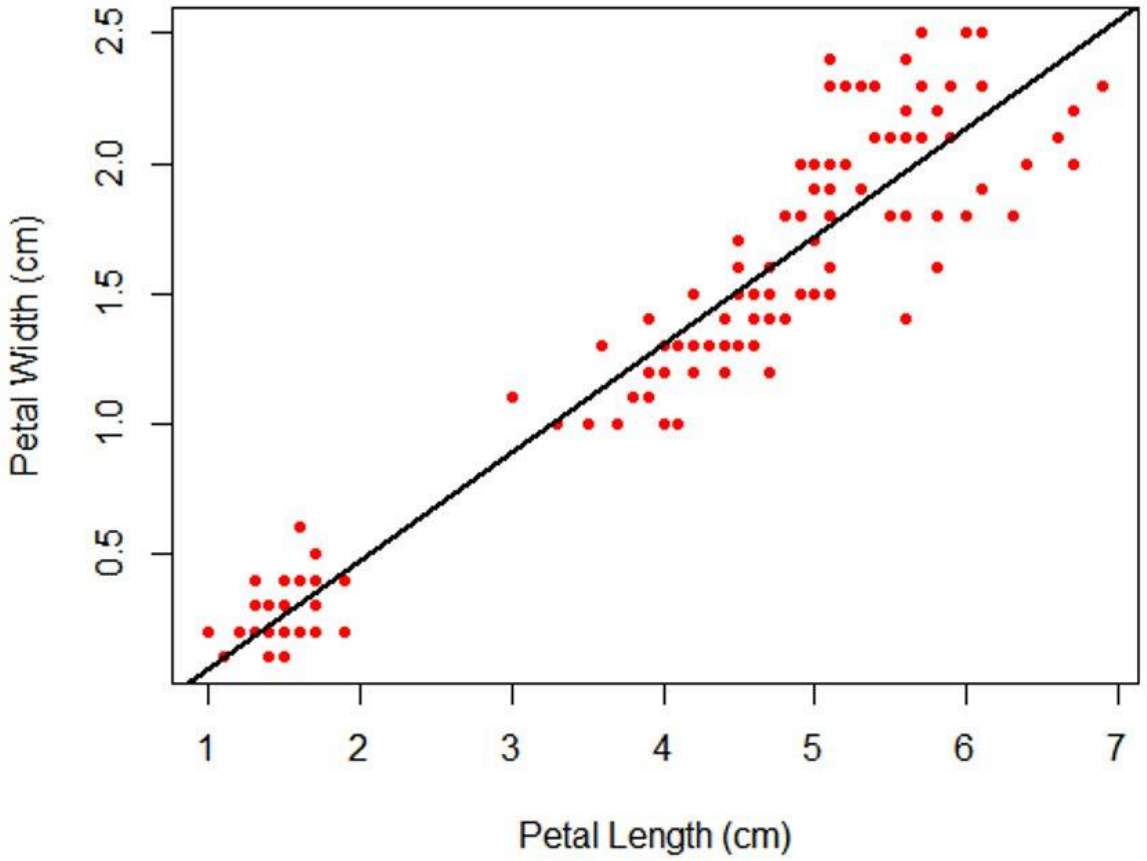
Scatter plot

```
plot(iris$Petal.Length, iris$Petal.Width,
       col="red", pch=20,
       xlab="Petal Length", ylab="Petal Width")
```



Scatter plot

```
plot(iris$Petal.Length, iris$Petal.Width,
       col="red", pch=20,
       xlab="Petal Length", ylab="Petal Width")
abline(lm(iris$Petal.Width~iris$Petal.Length),
       col="black", lwd=2)
```



Linear correlation

```
cor(iris$Petal.Length, iris$Petal.Width, method="pearson")
[1] 0.9628654

cor.test(iris$Petal.Length, iris$Petal.Width, method="pearson")

Pearson's product-moment correlation

data:  iris$Petal.Length and iris$Petal.Width
t = 43.387, df = 148, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.9490525 0.9729853
sample estimates:
      cor
0.9628654
```

Linear regression

Goal: Determine the extent to which there is a linear relationship between an "outcome" variable (dependent variable) and one more "explanatory" variables (independent variables, predictor variables).

Can a significant part of the variability in the outcome be predicted/explained by the independent variables?

Outcome variable: continuous (e.g. weight, heart rate, blood sugar)

Explanatory variables: continuous or (with adaptations) categorical

In R, the linear regression model is specified by a model formula of the form:

outcome ~ explanatory variables

Simple linear regression

A simple regression model (one explanatory variable) is specified by

$$y = a + b*x + err$$

a: Intercept

b: coefficient of explanatory var.

x: explanatory var.

err: error term (=residuals)

Assumptions :

- **Homoscedasticity** : independence between residual variance and variables
- Linearity + absence of linear relationship between predictor variables
- independence of the observations.
- **Residuals centered around predicted value** (mean=0)
- + normality of the **residual's mean**

  → only used to assess parameters confidence interval

Otherwise : try log-transform (for heteroskedasticity) or non-parametric methods if the assumptions are not met.

Summary of the data

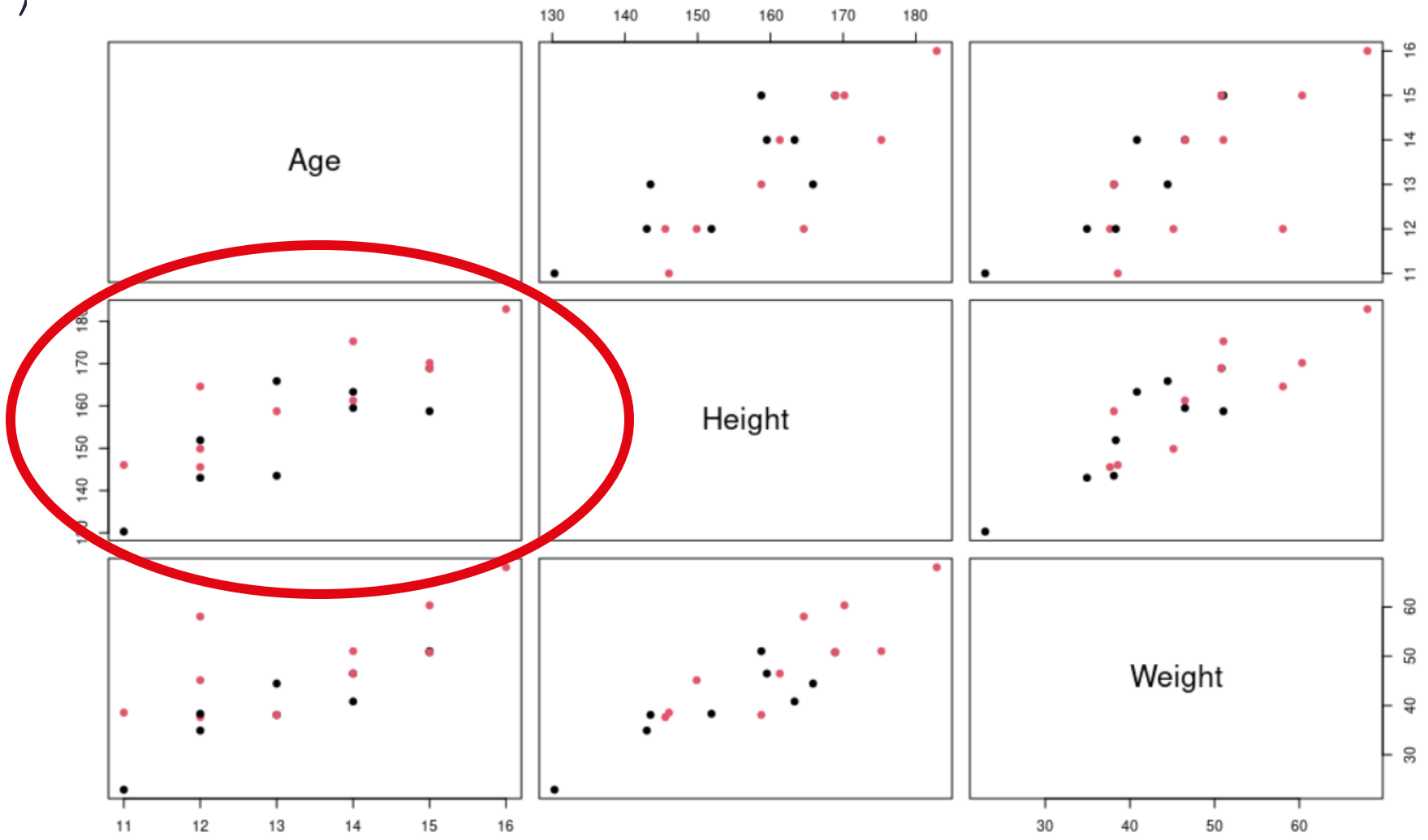
CLASS dataset, from the program SAS
(names removed and units have been modified from imperial to metric)

```
class_data <- read.csv("course_dataset/class.csv")
class_data$Gender=as.factor(class_data$Gender)
summary(class_data)
```

```
Gender       Age             Height          Weight
 F: 9   Min.   :11.00   Min.   :130.3   Min.   :22.91
 M:10   1st Qu.:12.00   1st Qu.:148.0   1st Qu.:38.22
        Median :13.00   Median :159.5   Median :45.13
        Mean   :13.32   Mean   :158.3   Mean   :45.37
        3rd Qu.:14.50   3rd Qu.:167.4   3rd Qu.:50.92
        Max.   :16.00   Max.   :182.9   Max.   :68.04
```

Visual summary of the data

```
pairs(class_data[2:4] , col = class_data$Gender,
    pch=19 )
```



The lm() function

lm() : fits a linear model.

- Creates an R object which contains the regression result
- Just printing the result provides only the regression coefficients.
- The **summary()** and **plot()** functions can be used to provide more information, including diagnostic plots.

Many other functions can be applied to the regression objects:

- **residuals()** extracts a vector containing the residuals (error)
- **coef()** extracts the regression coefficients
- **anova()** produces the corresponding ANOVA table (not covered

Simple linear regression code

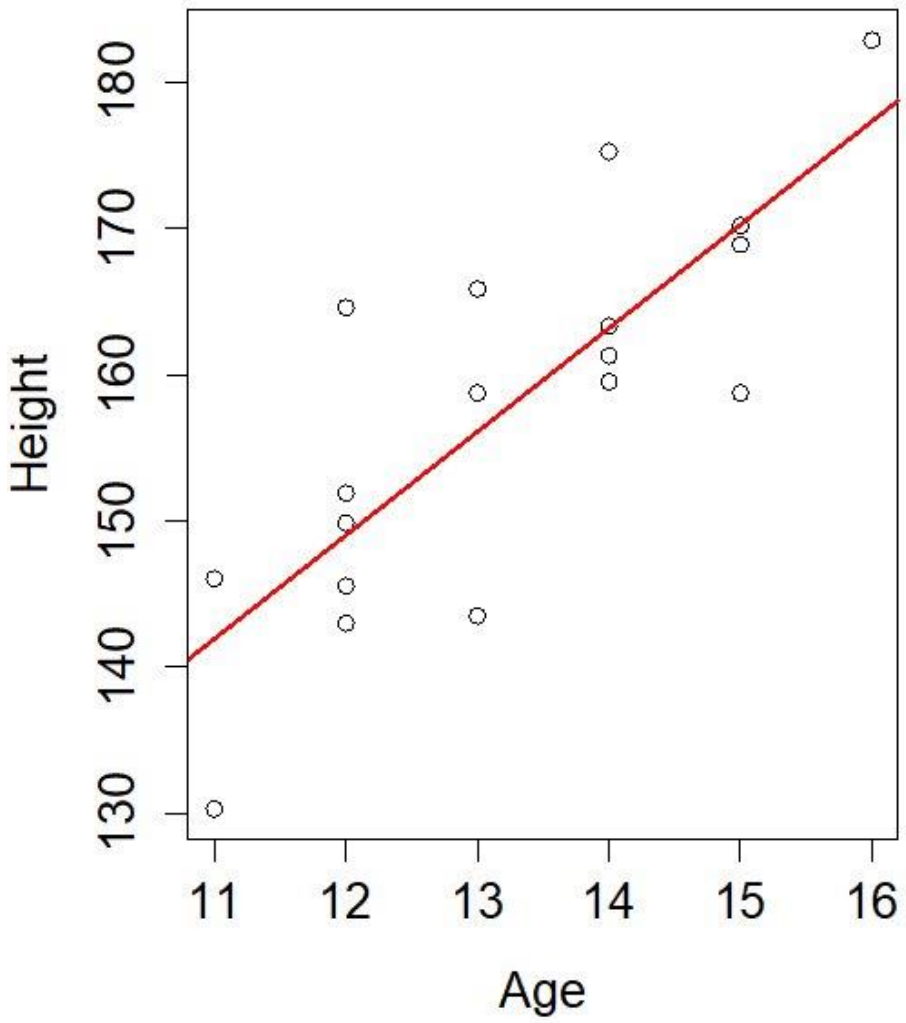
```
model_height_age <- lm(Height~Age, data=class_data)
model_height_age

Call:
lm(formula = Height ~ Age, data = class_data)

Coefficients:
(Intercept)          Age
     64.069        7.079
```

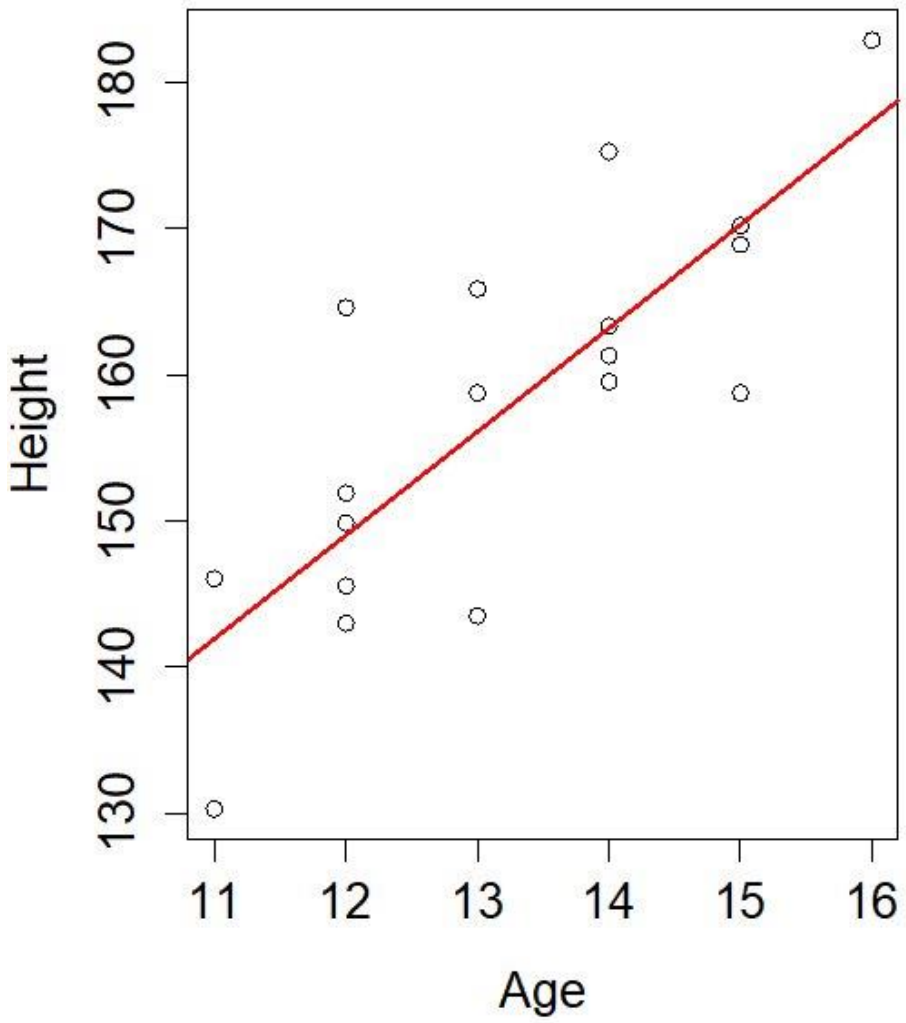
Representation of the fit (I)

```
plot(Height~Age,data=class_data)
abline(model_height_age, col="red", lwd=2)
```



Representation of the fit (II)

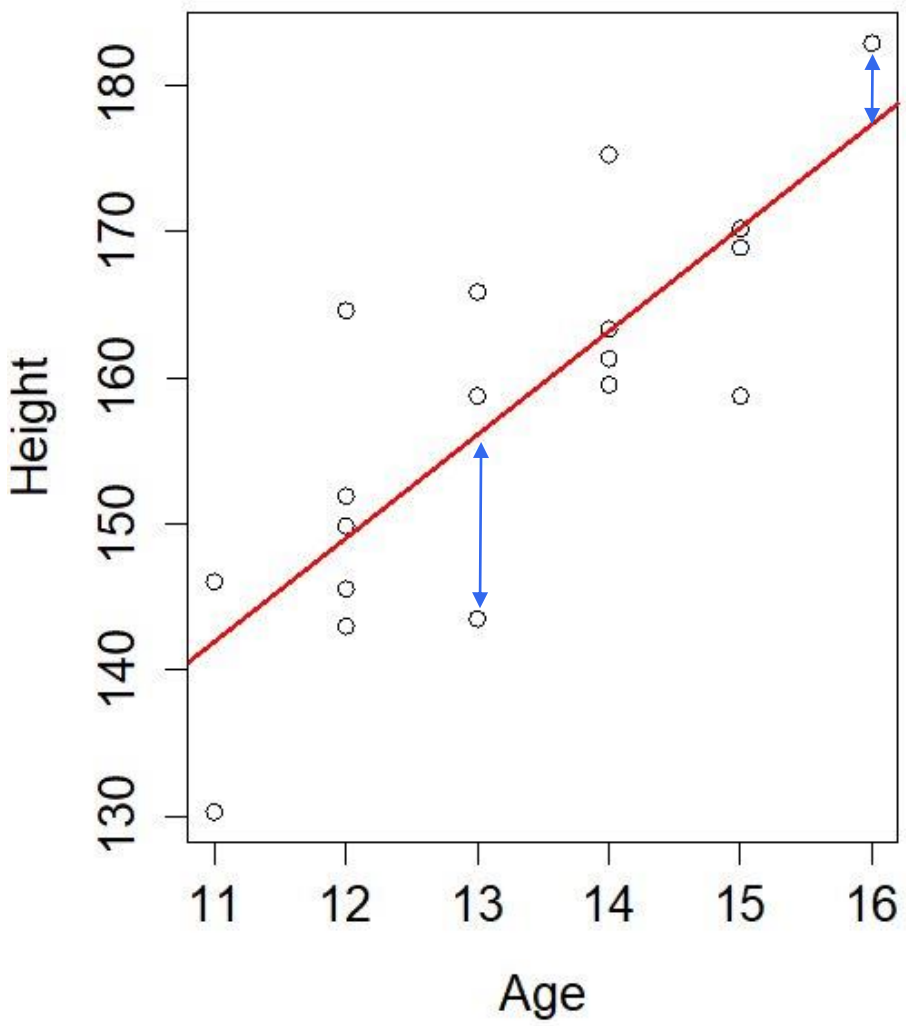
```
plot(Height~Age,data=class_data)
abline(model_height_age, col="red", lwd=2)
```



Coefficients: y-intercept and slope of the regression line

```
Coef(model_height_age)
(Intercept)    Age
64.068667       7.079333
```

Representation of the fit (III)



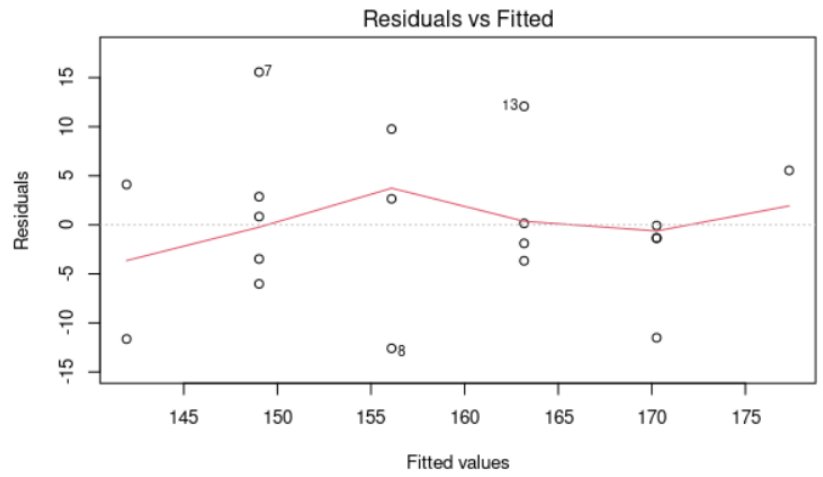
Residuals: vertical distances of data points from the regression line

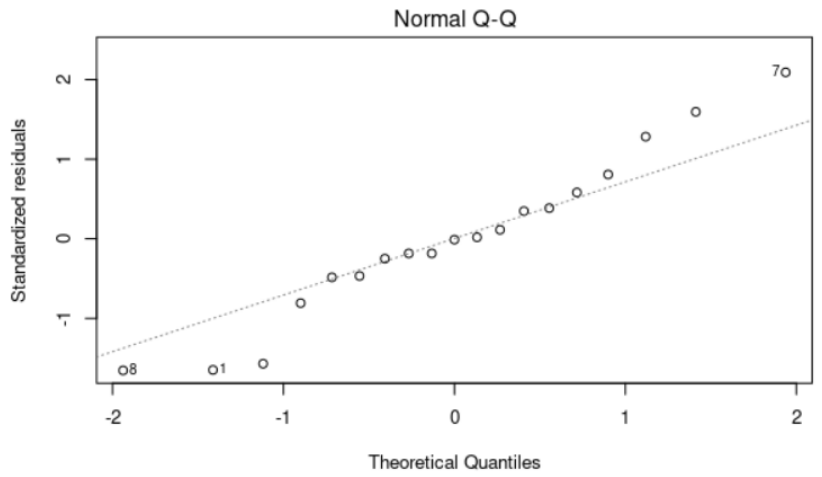
```
residuals(model_height_age)
          1           2           3           4
-11.63933333   4.10866667  -3.47866667   2.87133333
          5           6           7           8
  0.83933333  -6.01866667  15.57133333 -12.59000000
          9          10          11          12
  9.76200000   2.65000000  -3.66733333  -1.88933333
         13          14          15          16
12.08066667   0.14266667 -11.50866667  -1.34866667
         17          18          19
-0.07866667  -1.34866667   5.54200000
```

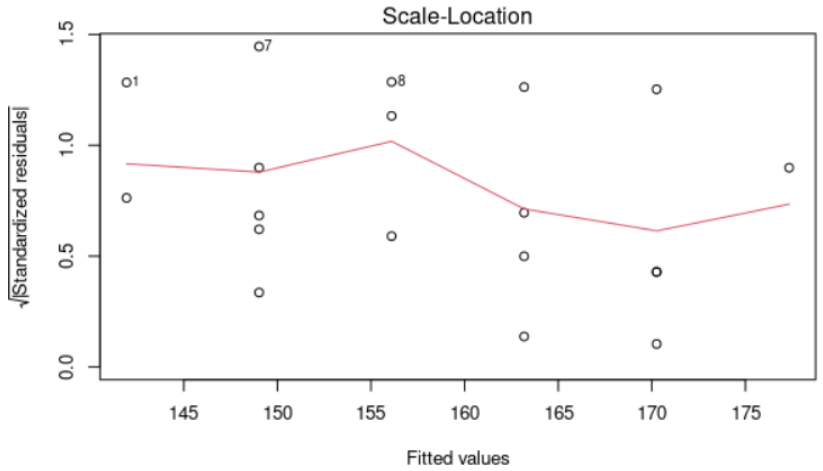
Check model assumptions

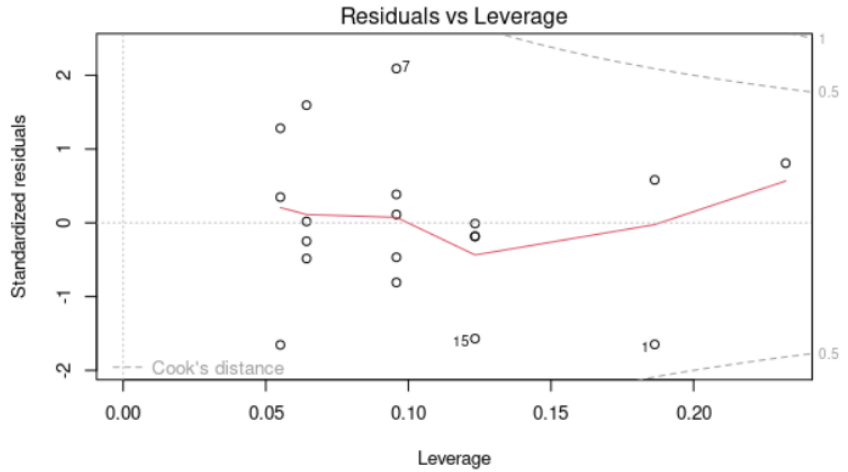
```
par(mfrow=c(2,2))
plot(model_height_age)
```

Commentaries on these plots:
https://library.virginia.edu/data/articles/diagnostic-plots







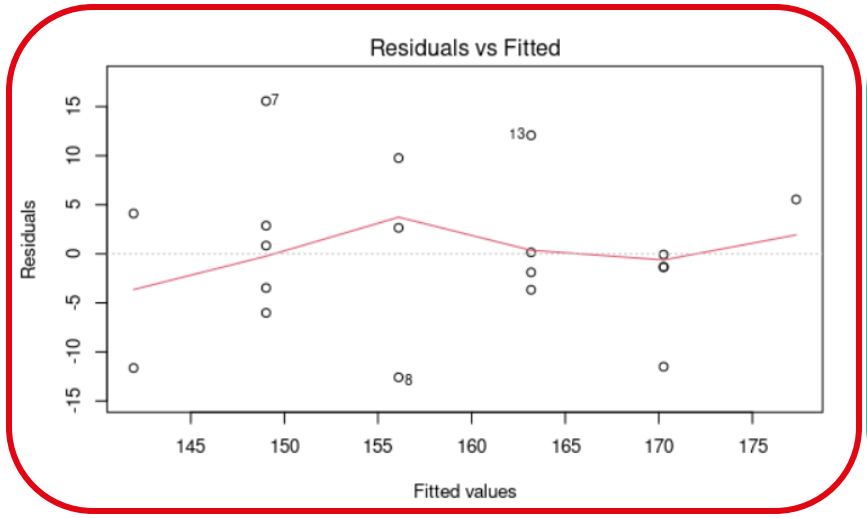


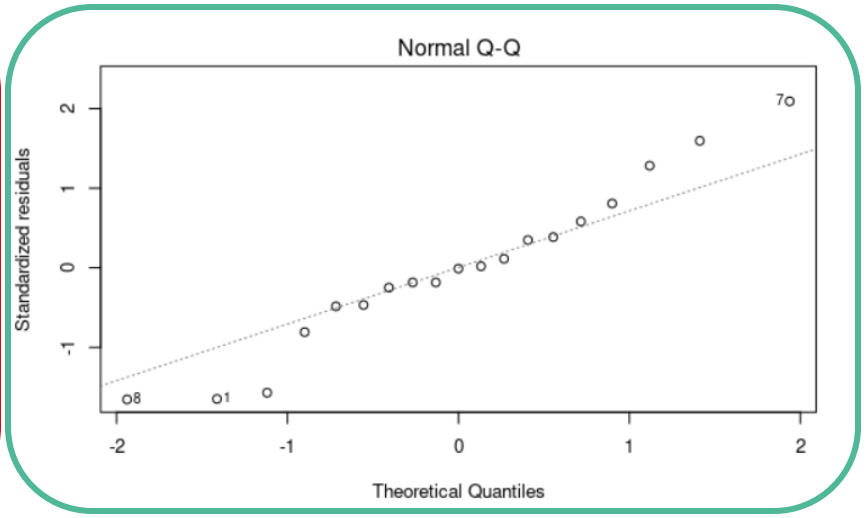
Check model assumptions

```
par(mfrow=c(2,2))
plot(model_height_age)
```

Commentaries on these plots:
https://library.virginia.edu/data/articles/diagnostic-plots

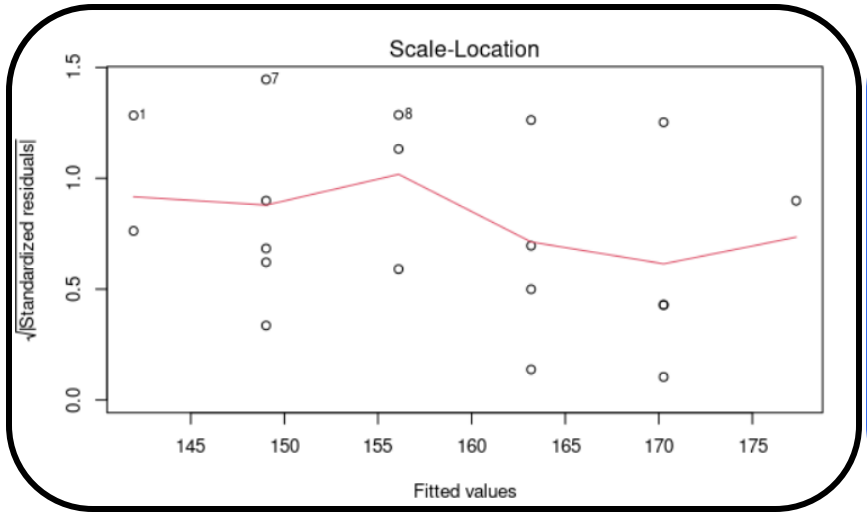
Residuals centered around 0

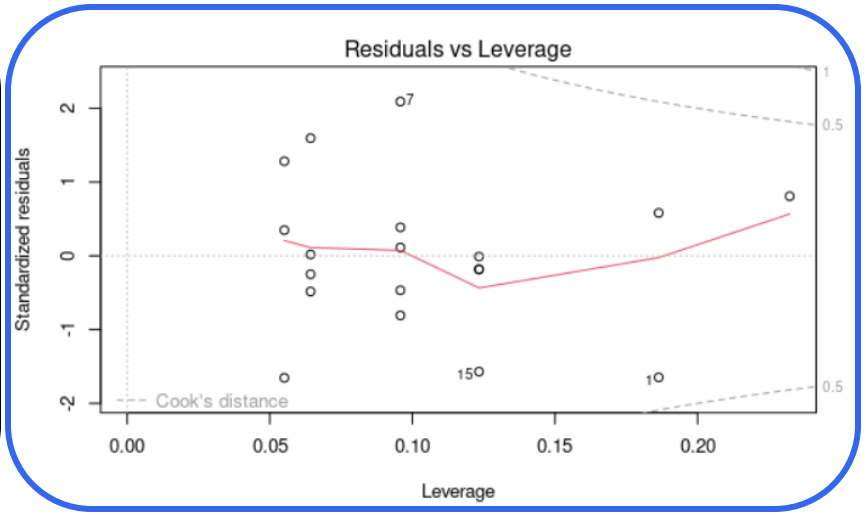




Normality of residuals

Homoskedascticity





Influence of points

Model summary

```
summary(model_height_age)
Call:
lm(formula = Height ~ Age, data = class_data)

Residuals:
     Min       1Q   Median       3Q      Max
-12.5900  -3.5730  -0.0787   3.4900  15.5713

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   64.069     16.565   3.868  0.00124 **
Age            7.079      1.237   5.724 2.48e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 7.832 on 17 degrees of freedom
Multiple R-squared:  0.6584, Adjusted R-squared:  0.6383
F-statistic: 32.77 on 1 and 17 DF,  p-value: 2.48e-05
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```

Residuals: difference between observed and fitted

Parameter significance:

- T-test
- Residual normality assumption

R-squared : fraction of the variance explained by the model

F-test: does the model explain significantly more than a model with just the intercept?

Let's practice - 11

The data set "Pima" comes from a study on diabetes in women of Pima Indian heritage.

We are using a subset (Pima.tr).

1. Load the package MASS using library(). Load the dataset Pima.tr using data(Pima.tr). Use ? to get an idea which variables it contains.

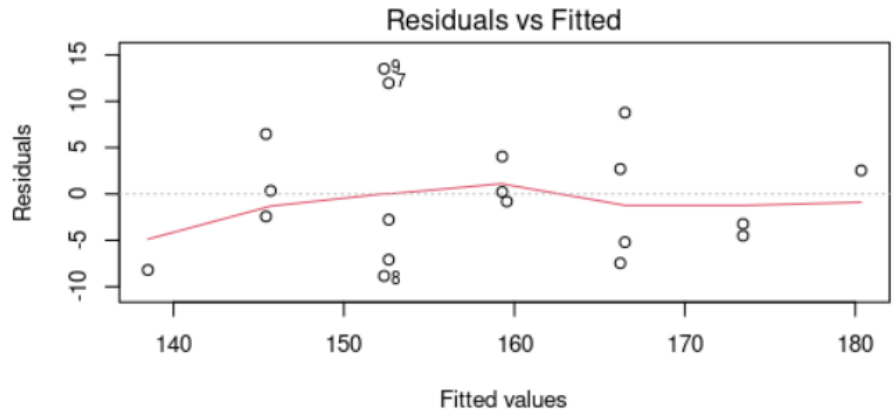
2. Hypothesis: Blood glucose level (glu) is associated with diastolic blood pressure (bp). Run a linear model to test the hypothesis.

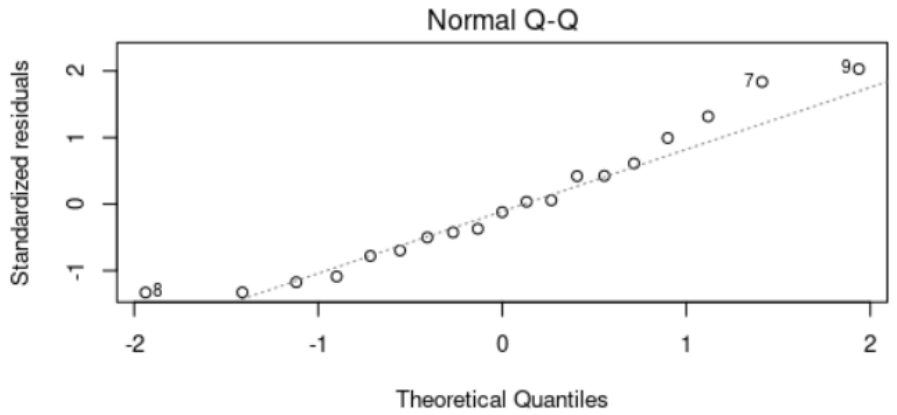
3. Visualize the fit with a scatter plot and a trend line.

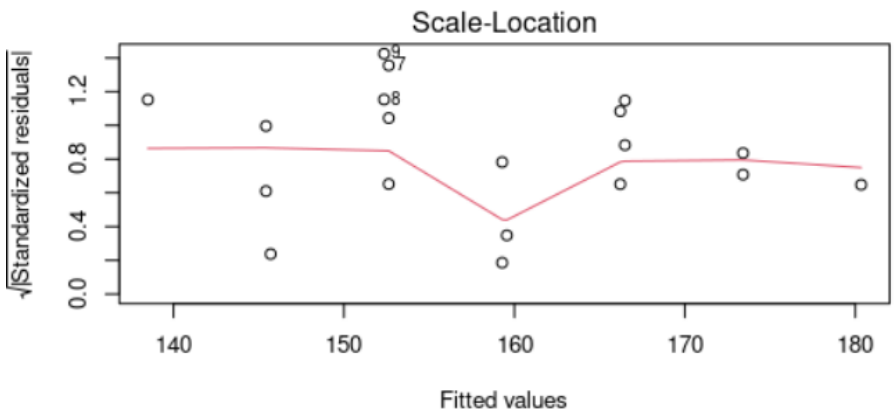
4. Check assumptions of the model (homoscedasticity, mean of residual at 0, normality of the residuals) graphically.

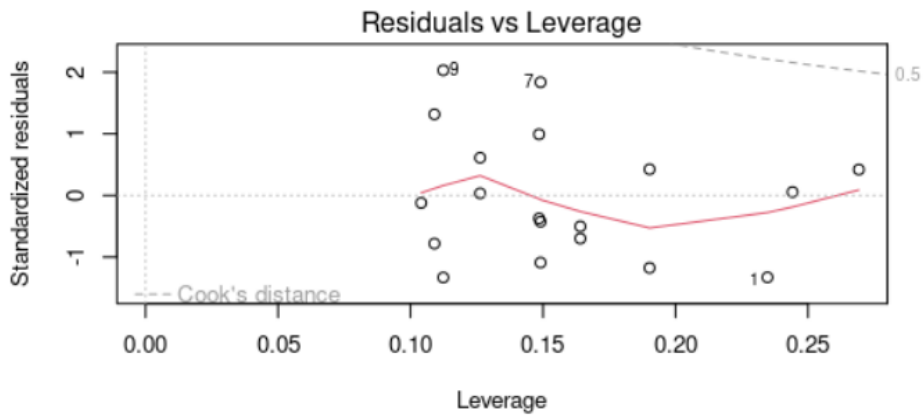
Additional content : model with 2 covariables (I)

```
model2 <- lm(Height~Age+Gender, data=class_data)
par(mfrow=c(2,2))
plot(model2)
```







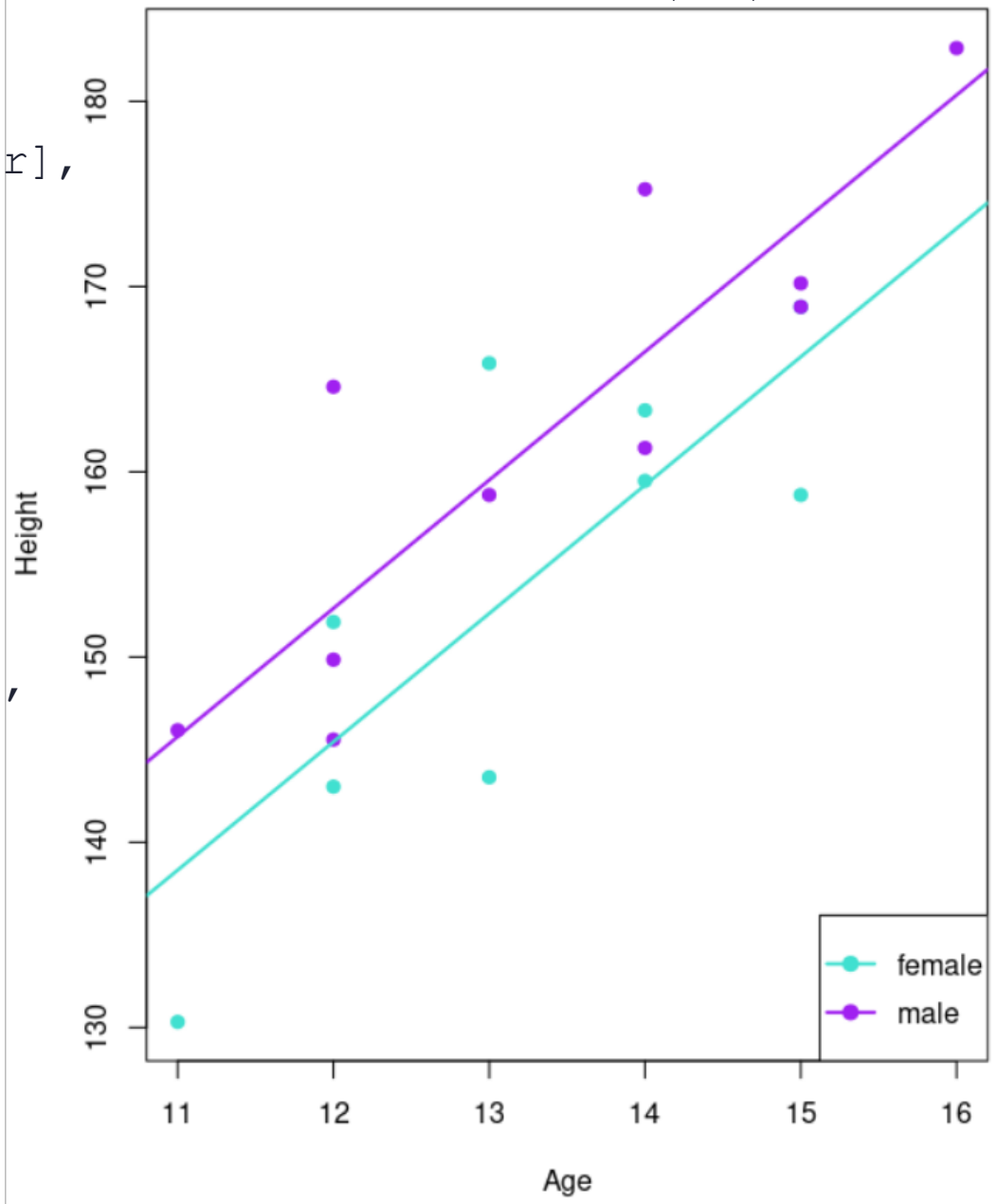


Additional content : model with 2 covariables (II)

```
summary( model2 )
Call:
lm(formula = Height ~ Age + Gender, data = class_data)

Residuals:
    Min      1Q  Median      3Q     Max
-8.8462 -4.8523 -0.8102  3.3677 13.5058

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   62.291     14.957   4.165 0.000731 ***
Age            6.928      1.117   6.202 1.27e-05 ***
GenderM        7.204      3.251   2.216 0.041517 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 7.061 on 16 degrees of freedom
Multiple R-squared:  0.7387,    Adjusted R-squared:  0.706
F-statistic: 22.61 on 2 and 16 DF,  p-value: 2.176e-05
```

Additional content : model with 2 covariables (III)

```
plot( Height~Age,
      col=c('turquoise','purple')[Gender],
      pch=19, data=class_data  )
C = coef( model2 )
abline(a=C[1],b=C[2] ,lwd=2,
       col='turquoise')
abline(a=C[1]+C[3],b=C[2] lwd=2,
       col='purple')
legend('bottomright',c('female','male'),
    pch=19,lwd=2,
    col=c('turquoise','purple'))
```



The next steps

- **R manuals:** http://cran.r-project.org/manuals.html
- **free course material:** https://glittr.org
- **STHDA (Statistical Tools for High Throughput Data Analysis) free tutorials:** http://www.sthda.com/english/
- **Stackoverflow** documentation, resources and user forum: http://stackoverflow.com/tags/r/info
- **Rseek -** search engine on numerous online R resources: http://www.rseek.org

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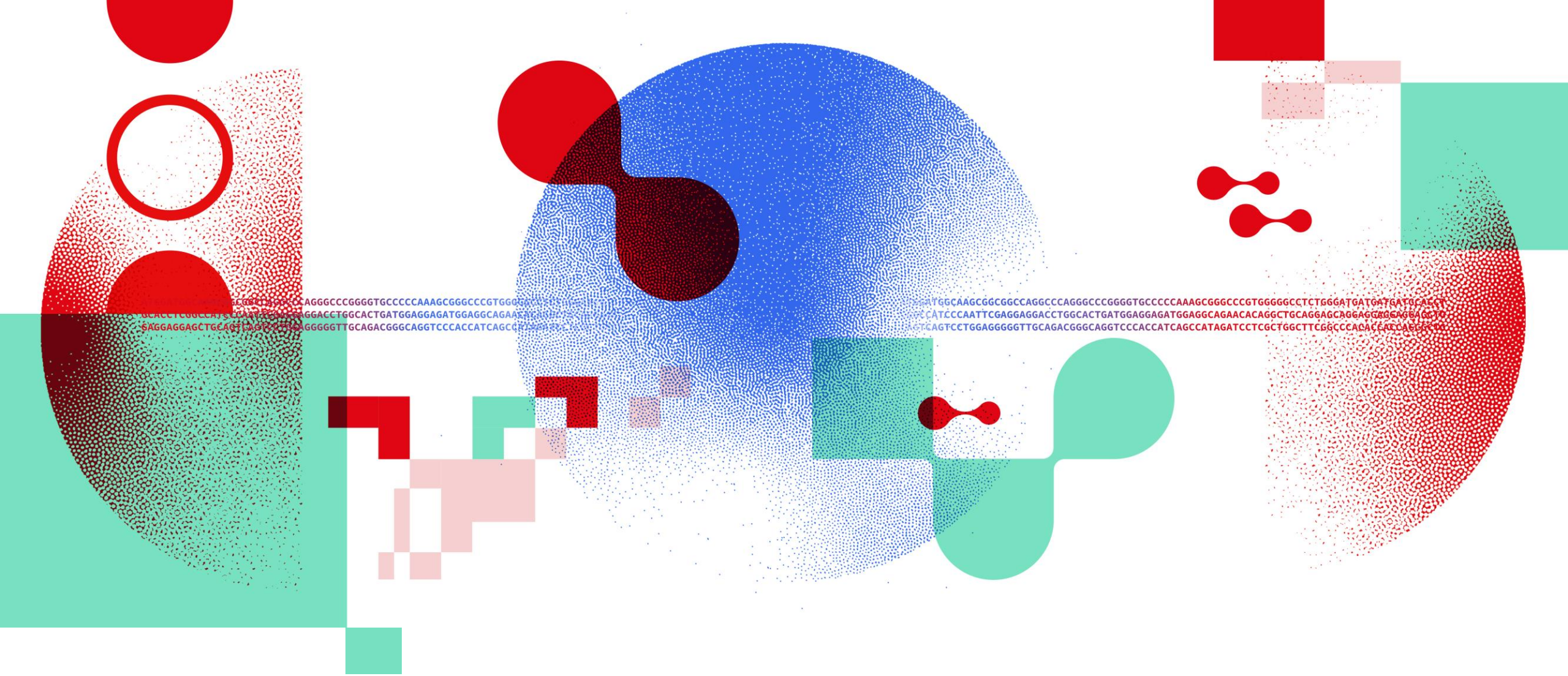
- Jenny DRNEVICH

http://www.sib.swiss/training

Any questions? Contact training@sib.swiss



Thank you

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