



CAMBRIDGE
INSTITUTE



UNIVERSITY OF
CAMBRIDGE

Introduction to Statistical Analysis

Cancer Research UK – 12th of February 2019

D.-L. Couturier / M. Eldridge / M. Fernandes [Bioinformatics core]

Timeline

9:30 – Morning

- ▶ ~ 45mn Lecture: data type, summary statistics and graphical displays
- ▶ ~ 15mn Quiz

10:30 – 15mn Coffee & Tea break

- ▶ ~ 60mn Lecture: some statistical distributions + CLT
- ▶ ~ 15mn Exercises & discussion

12:00 – Lunch break

13:00 – Afternoon

- ▶ ~ 45mn Lecture: One-sample location test
- ▶ ~ 30mn Exercises with shiny app & discussion

- ▶ ~ 45mn Lecture: Two-sample location test

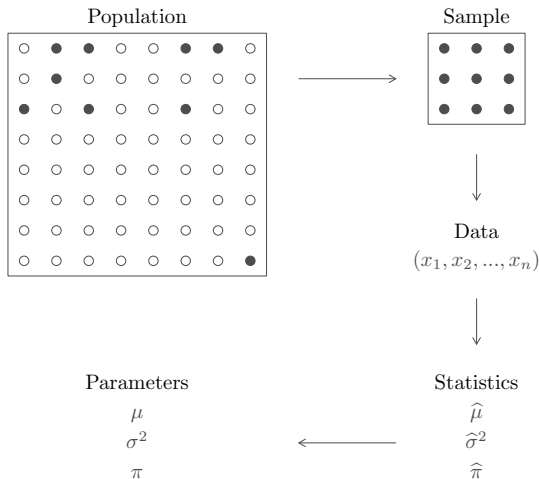
15:15 – 15mn Coffee & Tea break

- ▶ ~ 30mn Exercises with shiny app & discussion

16:15 – Group based exercises

- ▶ ~ 60mn

Grand Picture of Statistics



Data Types

	x_1	x_2	x_3	\dots	x_n
Cancer status	C	C	C	\dots	C
Nucleic acid sequence	C	T	T	\dots	A
5-level pain score	3	1	5	\dots	4
# of daily admissions at A&E	16	23	12	\dots	17
Gene expression intensity	882.1	379.5	528.3	\dots	120.9

Summary statistics and plots for qualitative data

5-level answers of 21 patients to the question

"How much did pain due to your ureteric stones interfere with your day to day activities ?":

3, 1, 5, 3, 1, 1, 1, 5, 1, 3, 4, 1, 1, 4, 5, 5, 5, 5, 5, 4, 4,

where

- ▶ 1 = "Not at all",
- ▶ 2 = "A little bit",
- ▶ 3 = "Somewhat",
- ▶ 4 = "Quite a bit",
- ▶ 5 = "Very much".

Summary statistics and plots for qualitative data

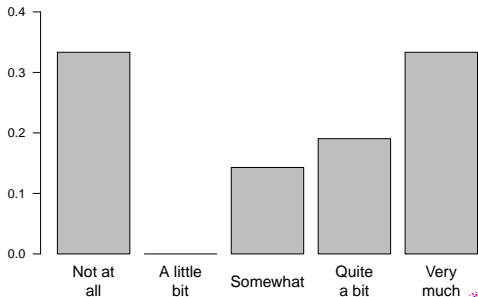
5-level answers of 21 patients to the question

"How much did pain due to your ureteric stones interfere with your day to day activities ?":

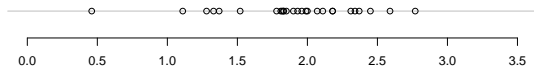
3, 1, 5, 3, 1, 1, 1, 5, 1, 3, 4, 1, 1, 4, 5, 5, 5, 5, 5, 4, 4,

where

- ▶ 1 = "Not at all",
- ▶ 2 = "A little bit",
- ▶ 3 = "Somewhat",
- ▶ 4 = "Quite a bit",
- ▶ 5 = "Very much".



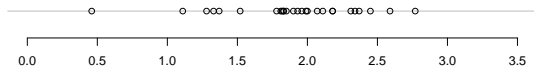
Summary statistics and plots for quantitative data



Gene expression values of gene “CCND3 Cyclin D3” from 27 patients diagnosed with acute lymphoblastic leukaemia:

$x_{(1)}$	$x_{(2)}$	$x_{(3)}$	$x_{(4)}$	$x_{(5)}$	$x_{(6)}$	$x_{(7)}$	$x_{(8)}$	$x_{(9)}$
0.46	1.11	1.28	1.33	1.37	1.52	1.78	1.81	1.82
$x_{(10)}$	$x_{(11)}$	$x_{(12)}$	$x_{(13)}$	$x_{(14)}$	$x_{(15)}$	$x_{(16)}$	$x_{(17)}$	$x_{(18)}$
1.83	1.83	1.85	1.9	1.93	1.96	1.99	2.00	2.07
$x_{(19)}$	$x_{(20)}$	$x_{(21)}$	$x_{(22)}$	$x_{(23)}$	$x_{(24)}$	$x_{(25)}$	$x_{(26)}$	$x_{(27)}$
2.11	2.18	2.18	2.31	2.34	2.37	2.45	2.59	2.77

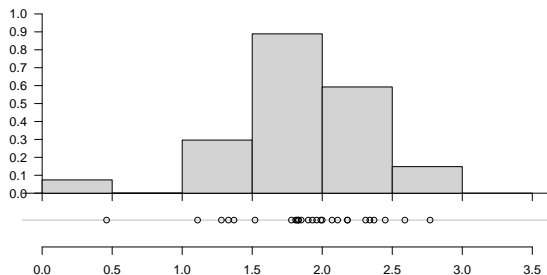
Summary statistics and plots for quantitative data



Gene expression values of gene “CCND3 Cyclin D3” from 27 patients diagnosed with acute lymphoblastic leukaemia:

$x_{(1)}$	$x_{(2)}$	$x_{(3)}$	$x_{(4)}$	$x_{(5)}$	$x_{(6)}$	$x_{(7)}$	$x_{(8)}$	$x_{(9)}$
0.46	1.11	1.28	1.33	1.37	1.52	1.78	1.81	1.82
$x_{(10)}$	$x_{(11)}$	$x_{(12)}$	$x_{(13)}$	$x_{(14)}$	$x_{(15)}$	$x_{(16)}$	$x_{(17)}$	$x_{(18)}$
1.83	1.83	1.85	1.9	1.93	1.96	1.99	2.00	2.07
$x_{(19)}$	$x_{(20)}$	$x_{(21)}$	$x_{(22)}$	$x_{(23)}$	$x_{(24)}$	$x_{(25)}$	$x_{(26)}$	$x_{(27)}$
2.11	2.18	2.18	2.31	2.34	2.37	2.45	2.59	2.77

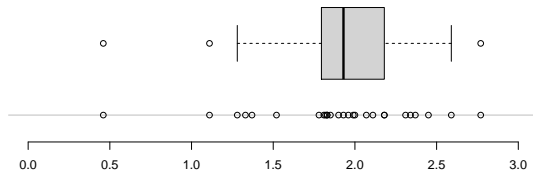
Summary statistics and plots for quantitative data



Gene expression values of gene “CCND3 Cyclin D3” from 27 patients diagnosed with acute lymphoblastic leukaemia:

$x_{(1)}$	$x_{(2)}$	$x_{(3)}$	$x_{(4)}$	$x_{(5)}$	$x_{(6)}$	$x_{(7)}$	$x_{(8)}$	$x_{(9)}$
0.46	1.11	1.28	1.33	1.37	1.52	1.78	1.81	1.82
$x_{(10)}$	$x_{(11)}$	$x_{(12)}$	$x_{(13)}$	$x_{(14)}$	$x_{(15)}$	$x_{(16)}$	$x_{(17)}$	$x_{(18)}$
1.83	1.83	1.85	1.9	1.93	1.96	1.99	2.00	2.07
$x_{(19)}$	$x_{(20)}$	$x_{(21)}$	$x_{(22)}$	$x_{(23)}$	$x_{(24)}$	$x_{(25)}$	$x_{(26)}$	$x_{(27)}$
2.11	2.18	2.18	2.31	2.34	2.37	2.45	2.59	2.77

Summary statistics and plots for quantative data



Gene expression values of gene “CCND3 Cyclin D3” from 27 patients diagnosed with acute lymphoblastic leukaemia:

$x_{(1)}$	$x_{(2)}$	$x_{(3)}$	$x_{(4)}$	$x_{(5)}$	$x_{(6)}$	$x_{(7)}$	$x_{(8)}$	$x_{(9)}$
0.46	1.11	1.28	1.33	1.37	1.52	1.78	1.81	1.82
$x_{(10)}$	$x_{(11)}$	$x_{(12)}$	$x_{(13)}$	$x_{(14)}$	$x_{(15)}$	$x_{(16)}$	$x_{(17)}$	$x_{(18)}$
1.83	1.83	1.85	1.9	1.93	1.96	1.99	2.00	2.07
$x_{(19)}$	$x_{(20)}$	$x_{(21)}$	$x_{(22)}$	$x_{(23)}$	$x_{(24)}$	$x_{(25)}$	$x_{(26)}$	$x_{(27)}$
2.11	2.18	2.18	2.31	2.34	2.37	2.45	2.59	2.77

Two-sample case: independent versus paired samples

Permeability constants of a placental membrane at term (X) and between 12 to 26 weeks gestational age (Y).

	1	2	3	4	5	6	7	8	9	10
X	0.80	0.83	1.89	1.04	1.45	1.38	1.91	1.64	0.73	1.46
Y	1.15	0.88	0.90	0.74	1.21					

Hamilton depression scale factor measurements in 9 patients with mixed anxiety and depression, taken at the first (X) and second (Y) visit after initiation of a therapy (administration of a tranquilizer).

	1	2	3	4	5	6	7	8	9
X	1.83	0.50	1.62	2.48	1.68	1.88	1.55	3.06	1.30
Y	0.88	0.65	0.60	2.05	1.06	1.29	1.06	3.14	1.29
Y-X	-0.95	0.15	-1.02	-0.43	-0.62	-0.59	-0.49	0.08	-0.01

Quiz Time

Sections 1 to 4

[https://docs.google.com/forms/d/
1C3RHisRHoWXcnFqX9JhRAk3gy_aJ6FrhouJ6ljsJ-Fc](https://docs.google.com/forms/d/1C3RHisRHoWXcnFqX9JhRAk3gy_aJ6FrhouJ6ljsJ-Fc)

Statistical distributions

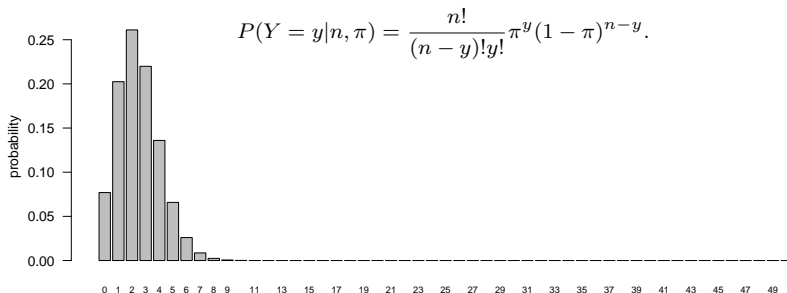
“In probability theory and statistics, a statistical distribution is a **mathematical function** that provides the **probabilities of occurrence of different possible outcomes** in an experiment” [Wikipedia].

Statistical distributions

“In probability theory and statistics, a statistical distribution is a **mathematical function** that provides the **probabilities of occurrence of different possible outcomes** in an experiment” [Wikipedia].

For a given cancer, mutation of the nucleic acid located at position 790 of Exon 20 is assumed to occur with a probability of $\sim 5\%$.

Probability of observing y patients out of $n = 50$ cancer patients with this mutation?



$$P(Y = y|n, \pi) = \frac{n!}{(n - y)!y!} \pi^y (1 - \pi)^{n-y}.$$

Some parametric distributions: Binomial distribution

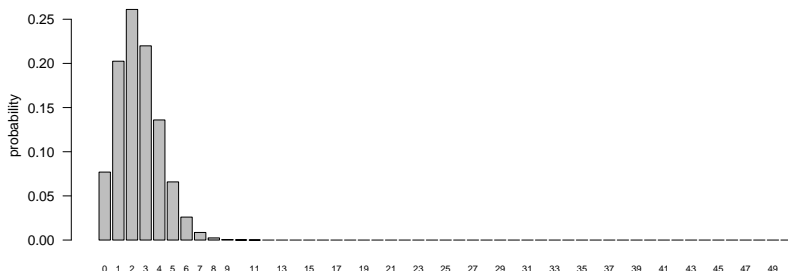
- ▶ the **number of successes out of n trials** (experiments), $Y = \sum_{i=1}^n X_i$, follows a binomial distribution with parameters n and π :

$$Y \sim \text{Bin}(n, \pi),$$

$$P(Y = y | n, \pi) = \frac{n!}{(n-y)!y!} \pi^y (1-\pi)^{n-y}.$$

IF

- ▶ n independent experiments,
- ▶ outcome of each experiment is dichotomous (success/failure),
- ▶ the probability of success π is the same for all experiments,



Some parametric distributions: Poisson distribution

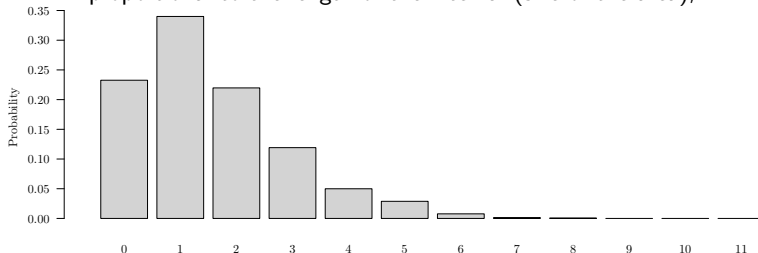
- ▶ the number of events occurring in a fixed time interval or in a given area, X , may be modelled by means of a Poisson distribution with parameter λ :

$$X \sim \text{Poisson}(\lambda),$$

$$P(X = x|\lambda) = \frac{\lambda^x e^{-\lambda}}{x!}.$$

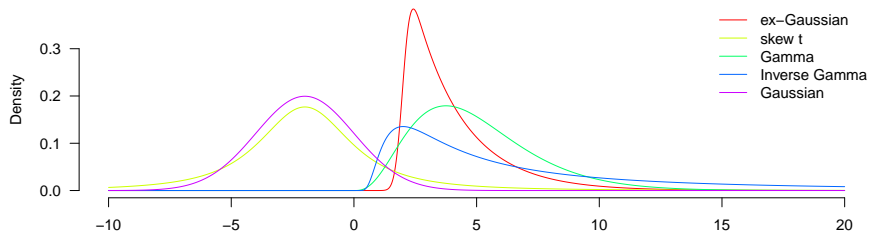
IF, during a time interval or in a given area,

- ▶ events occur independently,
- ▶ at the same rate,
- ▶ and the probability of an event to occur in a small interval (area) is proportional to the length of the interval (size of the area),



Number of chronic conditions per patient (US National Medical Expenditure Survey)

Some parametric distributions: Continuous distrib.



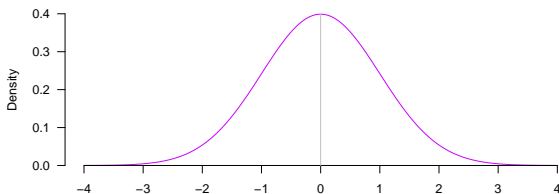
Some parametric distributions: Normal distribution

$$X \sim N(\mu, \sigma^2), \quad f_X(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$\mathbb{E}[X] = \mu, \quad \text{Var}[X] = \sigma^2,$$

$$Z = \frac{X - \mu}{\sigma} \sim N(0, 1), \quad f_Z(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}.$$

Probability density function, $f_Z(z)$, of a standard normal:



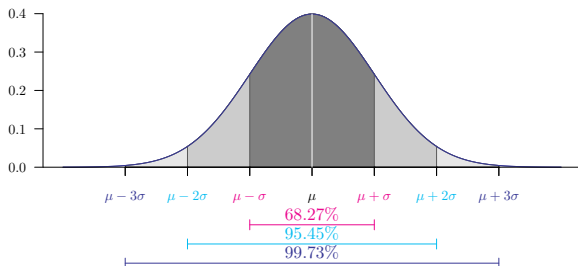
Some parametric distributions: Normal distribution

$$X \sim N(\mu, \sigma^2), \quad f_X(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$\mathbb{E}[X] = \mu, \quad \text{Var}[X] = \sigma^2,$$

$$Z = \frac{X - \mu}{\sigma} \sim N(0, 1), \quad f_Z(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}.$$

Probability density function, $f_Z(z)$, of a standard normal:



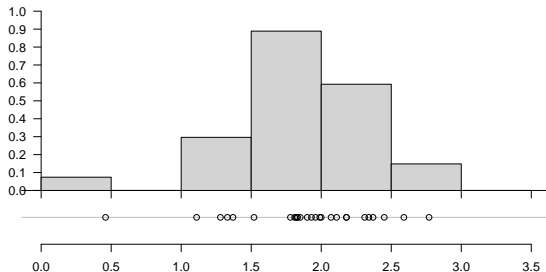
Some parametric distributions: Normal distribution

$$X \sim N(\mu, \sigma^2), \quad f_X(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$\mathbb{E}[X] = \mu, \quad \text{Var}[X] = \sigma^2,$$

$$Z = \frac{X - \mu}{\sigma} \sim N(0, 1), \quad f_Z(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}.$$

(i) Suitable modelling for a lot of variables



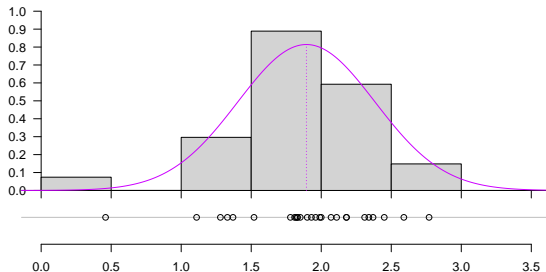
Some parametric distributions: Normal distribution

$$X \sim N(\mu, \sigma^2), \quad f_X(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$\mathbb{E}[X] = \mu, \quad \text{Var}[X] = \sigma^2,$$

$$Z = \frac{X - \mu}{\sigma} \sim N(0, 1), \quad f_Z(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}.$$

(i) Suitable modelling for a lot of variables



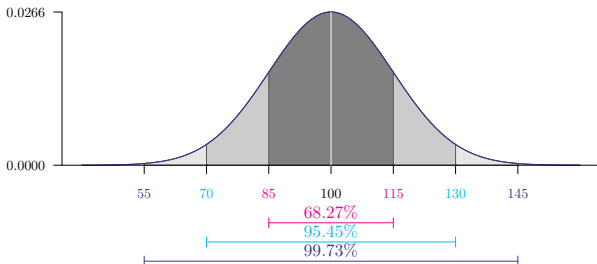
Some parametric distributions: Normal distribution

$$X \sim N(\mu, \sigma^2), \quad f_X(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$\mathbb{E}[X] = \mu, \quad \text{Var}[X] = \sigma^2,$$

$$Z = \frac{X - \mu}{\sigma} \sim N(0, 1), \quad f_Z(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}.$$

(i) Suitable modelling for a lot of variables: IQ



Some parametric distributions: Normal distribution

$$X \sim N(\mu, \sigma^2), \quad f_X(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

$$E[X] = \mu, \quad \text{Var}[X] = \sigma^2,$$

$$Z = \frac{X - \mu}{\sigma} \sim N(0, 1), \quad f_Z(z) = \frac{1}{\sqrt{2\pi}} e^{-\frac{z^2}{2}}.$$

(ii) Central limit theorem (Lindeberg-Lévy CLT)

- ▶ Let (X_1, \dots, X_n) be n independent and identically distributed (iid) random variables drawn from distributions of expected values given by μ and finite variances given by σ^2 ,
- ▶ then

$$\hat{\mu} = \overline{X} = \frac{\sum_{i=1}^n X_i}{n} \xrightarrow{d} N\left(\mu, \frac{\sigma^2}{n}\right).$$

If $X_i \sim N(\mu, \sigma^2)$, this result is true for all sample sizes.

Central limit theorem shiny app:

Distribution of the mean

<http://bioinformatics.cruk.cam.ac.uk/apps/stats/central-limit-theorem/>

95% Confidence interval for μ , the population mean,
when $X_i \sim N(\mu, \sigma^2)$

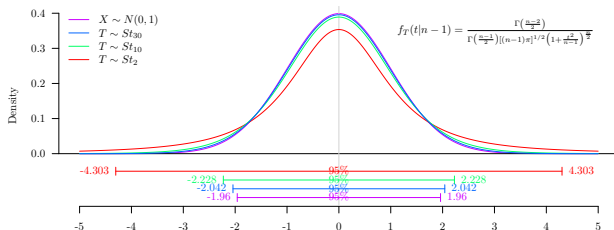
- ▶ if $X \sim N(\mu, \sigma^2)$, then $\bar{X} \sim N\left(\mu, \frac{\sigma^2}{n}\right)$,
- ▶ if $X \sim N(\mu, \sigma^2)$, then $Z = \frac{\bar{X} - \mu}{\frac{\sigma}{\sqrt{n}}} \sim N(0, 1)$,

$$P\left(- < - < \right) = 0.95$$

95% Confidence interval for μ , the population mean, when $X_i \sim N(\mu, \sigma^2)$

- ▶ if $X \sim N(\mu, \sigma^2)$, then $\bar{X} \sim N\left(\mu, \frac{\sigma^2}{n}\right)$,
- ▶ if $X \sim N(\mu, \sigma^2)$, then $Z = \frac{\bar{X} - \mu}{\sigma/\sqrt{n}} \sim N(0, 1)$,
- ▶ if σ unknown, then $T = \frac{\bar{X} - \mu}{s/\sqrt{n}} \sim St_{n-1}$.

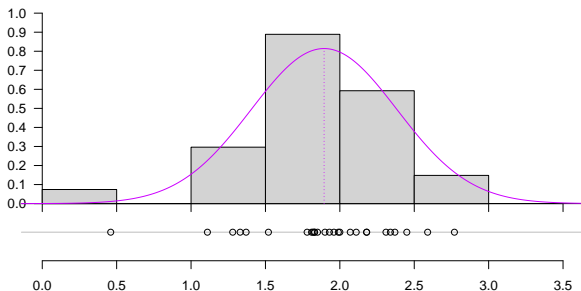
$$P\left(\bar{X} - \frac{s}{\sqrt{n}} \cdot t_{n-1, \alpha/2} < \mu < \bar{X} + \frac{s}{\sqrt{n}} \cdot t_{n-1, \alpha/2} \right) = 0.95$$



95% Confidence interval for μ , the population mean,
when $X_i \sim N(\mu, \sigma^2)$

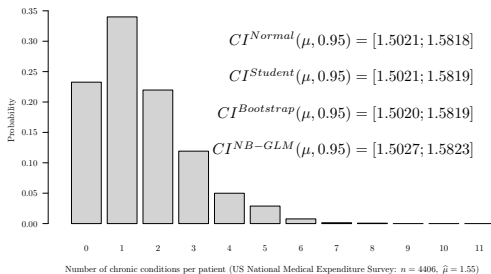
- ▶ if $X \sim N(\mu, \sigma^2)$, then $\bar{X} \sim N\left(\mu, \frac{\sigma^2}{n}\right)$,
- ▶ if $X \sim N(\mu, \sigma^2)$, then $Z = \frac{\bar{X} - \mu}{\frac{\sigma}{\sqrt{n}}} \sim N(0, 1)$,
- ▶ if σ unknown, then $T = \frac{\bar{X} - \mu}{\frac{s}{\sqrt{n}}} \sim St_{n-1}$.

$$P\left(\quad < \quad < \quad \right) = 0.95$$



95% Confidence interval for μ , the population mean, when $X_i \sim iid(\mu, \sigma^2)$

- ▶ CLT: $\bar{X} \xrightarrow{d} N\left(\mu, \frac{\sigma^2}{n}\right)$,
- ▶ if $X \sim N(\mu, \sigma^2)$, then $Z = \frac{X-\mu}{\sigma} \sim N(0, 1)$,
- ▶ if σ unknown, then $T = \frac{X-\mu}{s} \sim St_{n-1}$.



95% Confidence interval for $\mu_Y - \mu_X$, the difference between population means

If we have

- ▶ $X_i \sim iid(\mu_X, \sigma_X^2), i = 1, \dots, n_X,$
- ▶ $Y_i \sim iid(\mu_Y, \sigma_Y^2), i = 1, \dots, n_Y,$

95% Confidence interval for $\mu_Y - \mu_X$, the difference between population means

If we have

- ▶ $X_i \sim iid(\mu_X, \sigma_X^2), i = 1, \dots, n_X,$
- ▶ $Y_i \sim iid(\mu_Y, \sigma_Y^2), i = 1, \dots, n_Y,$

then

- ▶ if $\sigma_X^2 = \sigma_Y^2$ [Student's t-test equation],

$$\triangleright CI(\mu_Y - \mu_X, 0.95) = (\bar{Y} - \bar{X}) \pm t_{1-\frac{\alpha}{2}, n_X+n_Y-2} s_p \sqrt{\frac{1}{n_X} + \frac{1}{n_Y}}$$

$$\text{where } s_p = \frac{(n_X-1)s_X^2 + (n_Y-1)s_Y^2}{n_X+n_Y-2},$$

95% Confidence interval for $\mu_Y - \mu_X$, the difference between population means

If we have

- ▶ $X_i \sim iid(\mu_X, \sigma_X^2)$, $i = 1, \dots, n_X$,
- ▶ $Y_i \sim iid(\mu_Y, \sigma_Y^2)$, $i = 1, \dots, n_Y$,

then

- ▶ if $\sigma_X^2 = \sigma_Y^2$ [Student's t-test equation],

$$\triangleright CI(\mu_Y - \mu_X, 0.95) = (\bar{Y} - \bar{X}) \pm t_{1-\frac{\alpha}{2}, n_X+n_Y-2} s_p \sqrt{\frac{1}{n_X} + \frac{1}{n_Y}}$$

$$\text{where } s_p = \frac{(n_X-1)s_X^2 + (n_Y-1)s_Y^2}{n_X+n_Y-2},$$

- ▶ if $\sigma_X^2 \neq \sigma_Y^2$ [Welch-Satterthwaite's t-test equation],

$$\triangleright CI(\mu_Y - \mu_X, 0.95) = (\bar{Y} - \bar{X}) \pm t_{1-\frac{\alpha}{2}, df} \sqrt{\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y}}, \text{ where}$$

$$df = \frac{\left(\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y}\right)^2}{\frac{\left(\frac{s_X^2}{n_X}\right)^2}{n_X-1} + \frac{\left(\frac{s_Y^2}{n_Y}\right)^2}{n_Y-1}}.$$

Central limit theorem shiny app:

Coverage of Student's asymptotic confidence intervals

<http://bioinformatics.cruk.cam.ac.uk/apps/stats/central-limit-theorem/>

Quiz Time

Practical 1

`http://bioinformatics-core-shared-training.github.io/
IntroductionToStats/practical.html`

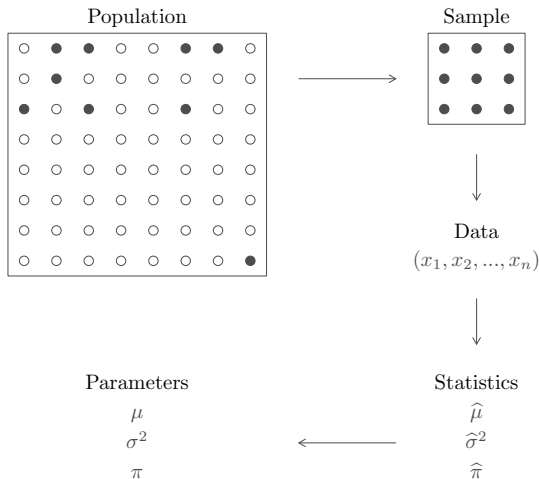
PART II:

Parametric and non-parametric one-sample location tests

Cancer Research UK – 12th of February 2019

D.-L. Couturier / M. Eldridge / M. Fernandes [Bioinformatics core]

Grand Picture of Statistics



Statistical hypothesis testing

A hypothesis test describes a phenomenon by means of two non-overlapping idealised models/descriptions:

- ▶ the null hypothesis **H0**, “generally assumed to be true until evidence indicates otherwise”
- ▶ the alternative hypothesis **H1**.

The aim of the test is to reject the null hypothesis in favour of the alternative hypothesis, and conclude, with a probability α of being wrong, that the idealised model/description of H1 is true.

Theory 1: Dieters lose more fat than the exercisers

Theory 2: There is no majority for Brexit now

Theory 3: Serum vitamin C is reduced in patients

Statistical hypothesis testing

Several-step process:

- ▶ Define H_0 and H_1 according to a theory
- ▶ Set α , the probability of rejecting H_0 when it is true (type I error),
- ▶ Define n , the sample size, allowing you to reject H_0 when H_1 is true with a probability $1 - \beta$ (Power),
- ▶ Determine the test statistic to be used,
- ▶ Collect the data,
- ▶ Perform the statistical test, define the p -value, and reject (or not) the null hypothesis.

Statistical hypothesis testing

Many options:

- ▶ One-sided versus two-sided tests,
- ▶ Exact versus asymptotic tests,
- ▶ Parametric versus non-parametric tests.

Parametric or non-parametric ?

T-test		Outcome(s) normally distributed		
		Yes	Mildly	No
Sample size	Small			
	Medium			
	Large			

Situations which may suggest the use of non-parametric statistics:

- ▶ When there is a small sample size or **very unequal groups**,
- ▶ When the data has **notable outliers**,
- ▶ When one outcome has a **distribution other than normal**,
- ▶ When the data are **ordered** with many ties or are rank ordered.

Parametric location test

(One-sided) t-test

We test:

$$H_0: \mu_{IQ} = 100,$$

$$H_1: \mu_{IQ} > 100.$$

We have $X_i \sim N(\mu, \sigma^2), i = 1, \dots, n,$

We know

$$\blacktriangleright \bar{X} \sim N\left(\mu, \frac{\sigma^2}{n}\right),$$

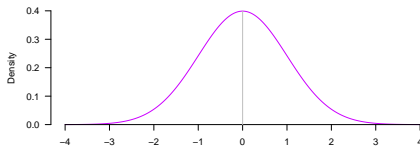
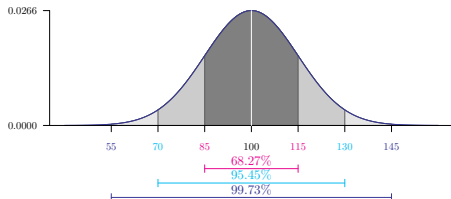
$$\blacktriangleright Z = \frac{\bar{X} - \mu}{\frac{\sigma}{\sqrt{n}}} \sim N(0, 1),$$

Thus, if H_0 is true, we have:

$$\blacktriangleright Z = \frac{\bar{X} - \mu_0}{\frac{\sigma}{\sqrt{n}}} \sim N(0, 1).$$

Define the p-value:

$$\blacktriangleright p\text{-value} = P(T > T_{obs})$$



Statistical hypothesis testing

4 possible outcomes

Conclude:

- ▶ if $p\text{-value} > \alpha \rightarrow$ do not reject H_0 .
- ▶ if $p\text{-value} < \alpha \rightarrow$ reject H_0 in favour of H_1 .

		Test Outcome	
		H0 not rejected	H1 accepted
Unknown Truth	H0 true	$1 - \alpha$	α
	H1 true	β	$1 - \beta$

where

- ▶ α is the type I error,
- ▶ β is the type II error.

Parametric location test

(One-sided) binomial exact test

We test:

H0: $\pi = 5\%$,

H1: $\pi > 5\%$.

We have $X_i \sim \text{Bernoulli}(\pi), i = 1, \dots, n$,

We know

$$\blacktriangleright Y = \sum_{i=1}^n X_i \sim \text{Binomial}(\pi, n),$$

Thus, if H0 is true, we have:

$$\blacktriangleright Y = \sum_{i=1}^n X_i \sim \text{Binomial}(5\%, n),$$

Parametric location test

(One-sided) binomial exact test

We test:

H0: $\pi = 5\%$,

H1: $\pi > 5\%$.

We have $X_i \sim \text{Bernoulli}(\pi), i = 1, \dots, n$,

We know

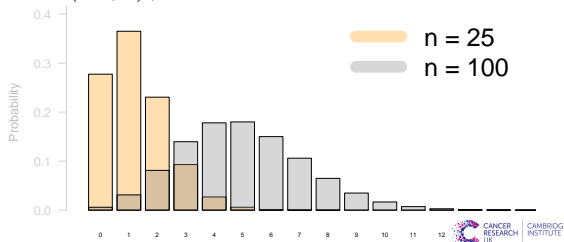
$$\blacktriangleright Y = \sum_{i=1}^n X_i \sim \text{Binomial}(\pi, n),$$

Thus, if H0 is true, we have:

$$\blacktriangleright Y = \sum_{i=1}^n X_i \sim \text{Binomial}(5\%, n),$$

Define the p-value:

$$\blacktriangleright p\text{-value} = P(Y > Y_{obs})$$



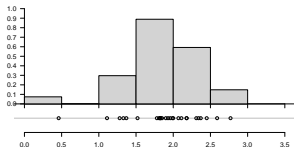
Non-parametric location test

Wilcoxon sign-rank test

A location model is assumed for X_i , $i = 1, \dots, n$:

$$X_i = \theta + e_i,$$

where $e_i \sim iid(\mu_e = 0, \sigma_e^2)$, a symmetrical distribution.



Interest for **H0**: $\theta = \theta_0$ against **H1**: $\theta < \theta_0$ or $\theta \neq \theta_0$ or $\theta > \theta_0$.

Test statistics : $W^+ = \sum_{i=1}^n \iota(X_i - \theta_0 > 0) \text{ Rank}(|X_i - \theta_0|)$.

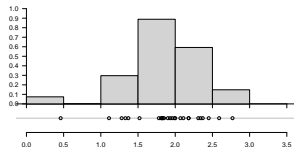
Non-parametric location test

Wilcoxon sign-rank test

A location model is assumed for X_i , $i = 1, \dots, n$:

$$X_i = \theta + e_i,$$

where $e_i \sim iid(\mu_e = 0, \sigma_e^2)$, a symmetrical distribution.



Interest for **H0**: $\theta = \theta_0$ against **H1**: $\theta < \theta_0$ or $\theta \neq \theta_0$ or $\theta > \theta_0$.

Test statistics : $W^+ = \sum_{i=1}^n \iota(X_i - \theta_0 > 0) \text{Rank}(|X_i - \theta_0|)$.

Distribution of W under H0: W^+ has no closed-form distribution.

Wilcoxon signed rank test

```
data: golub[1042, gol.fac == "ALL"]
V = 268, p-value = 0.05847
alternative hypothesis: true location is not equal to 1.75
95 percent confidence interval:
 1.73868 2.09106
sample estimates:
(pseudo)median
 1.926475
```

Introduction to Shiny Apps and Exercises

PART III:

Parametric and non-parametric two-sample location tests

Cancer Research UK – 12th of February 2019

D.-L. Couturier / M. Eldridge / M. Fernandes [Bioinformatics core]

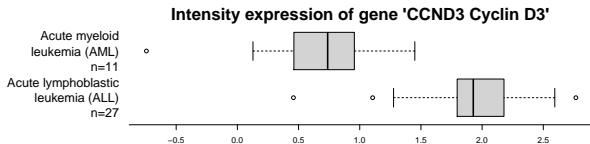
Two-sample case

Many options:

- ▶ One-sided versus two-sided tests,
- ▶ Exact versus asymptotic tests,
- ▶ Parametric versus non-parametric tests,
- ▶ Tests for paired versus independent data.

Parametric two-sample location test

Two-sample two-sided Student-s & Welch's t-tests



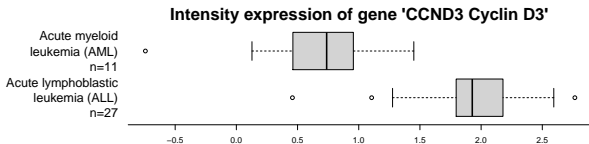
We test $H_0: \mu_Y - \mu_X = 0$ against $H_1: \mu_Y - \mu_X \neq 0$.

We know:

- ▶ Student's t-test [assume $\sigma_X^2 = \sigma_Y^2$]:
$$\frac{(\bar{Y} - \bar{X}) - (\mu_Y - \mu_X)}{s_p \sqrt{\frac{1}{n_X} + \frac{1}{n_Y}}} \sim t_{1 - \frac{\alpha}{2}, n_X + n_Y - 2}$$
- ▶ Welch's t-test [assume $\sigma_X^2 \neq \sigma_Y^2$]:
$$\frac{(\bar{Y} - \bar{X}) - (\mu_Y - \mu_X)}{\sqrt{\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y}}} \sim t_{1 - \frac{\alpha}{2}, df}$$

Parametric two-sample location test

Two-sample two-sided Student-s & Welch's t-tests



We test $H_0: \mu_Y - \mu_X = 0$ against $H_1: \mu_Y - \mu_X \neq 0$.

We know:

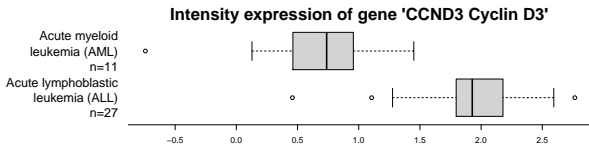
- ▶ Student's t-test [assume $\sigma_X^2 = \sigma_Y^2$]: $\frac{(\bar{Y} - \bar{X}) - (\mu_Y - \mu_X)}{s_p \sqrt{\frac{1}{n_X} + \frac{1}{n_Y}}} \sim t_{1-\frac{\alpha}{2}, n_X + n_Y - 2}$
- ▶ Welch's t-test [assume $\sigma_X^2 \neq \sigma_Y^2$]: $\frac{(\bar{Y} - \bar{X}) - (\mu_Y - \mu_X)}{\sqrt{\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y}}} \sim t_{1-\frac{\alpha}{2}, df}$

Two Sample t-test

```
data: golub[1042, gol.fac == "ALL"] and golub[1042, gol.fac == "AML"]
t = 6.7983, df = 36, p-value = 6.046e-08
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.8829143 1.6336690
sample estimates:
mean of x mean of y
1.8938826 0.6355909
```

Parametric two-sample location test

Two-sample two-sided Student-s & Welch's t-tests



We test $H_0: \mu_Y - \mu_X = 0$ against $H_1: \mu_Y - \mu_X \neq 0$.

We know:

- ▶ Student's t-test [assume $\sigma_X^2 = \sigma_Y^2$]: $\frac{(\bar{Y} - \bar{X}) - (\mu_Y - \mu_X)}{s_p \sqrt{\frac{1}{n_X} + \frac{1}{n_Y}}} \sim t_{1 - \frac{\alpha}{2}, n_X + n_Y - 2}$
- ▶ Welch's t-test [assume $\sigma_X^2 \neq \sigma_Y^2$]: $\frac{(\bar{Y} - \bar{X}) - (\mu_Y - \mu_X)}{\sqrt{\frac{s_X^2}{n_X} + \frac{s_Y^2}{n_Y}}} \sim t_{1 - \frac{\alpha}{2}, df}$

Welch Two Sample t-test

```
data: golub[1042, gol.fac == "ALL"] and golub[1042, gol.fac == "AML"]
t = 6.3186, df = 16.118, p-value = 9.871e-06
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.8363826 1.6802008
sample estimates:
mean of x mean of y
1.8938826 0.6355909
```

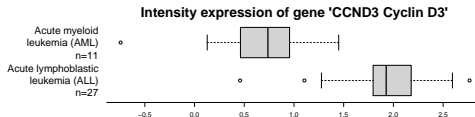
Non-parametric two-sample location test

Mann-Whitney-Wilcoxon test

Let

► $X_i \sim iid(\mu_X, \sigma^2), i = 1, \dots, n_X,$

► $Y_i \sim iid(\mu_X + \delta, \sigma^2), i = 1, \dots, n_Y.$



Interest for **H0**: $\delta = \delta_0$ against **H1**: $\delta < \delta_0$ or $\delta \neq \delta_0$ or $\delta > \delta_0$.

Standardised test statistic: $z = \frac{\sum_{i=1}^{n_Y} R(Y_i) - [n_Y(n_X + n_Y + 1)/2]}{\sqrt{n_X n_Y (n_X + n_Y + 1)/12}},$

where $R(Y_i)$ denotes the rank of Y_i amongst the combined samples, i.e., amongst $(X_1, \dots, X_{n_X}, Y_1, \dots, Y_{n_Y})$.

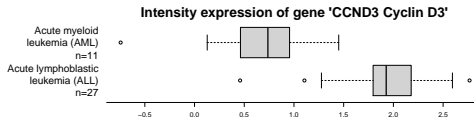
Non-parametric two-sample location test

Mann-Whitney-Wilcoxon test

Let

► $X_i \sim iid(\mu_X, \sigma^2), i = 1, \dots, n_X,$

► $Y_i \sim iid(\mu_X + \delta, \sigma^2), i = 1, \dots, n_Y.$



Interest for **H0**: $\delta = \delta_0$ against **H1**: $\delta < \delta_0$ or $\delta \neq \delta_0$ or $\delta > \delta_0$.

Standardised test statistic: $z = \frac{\sum_{i=1}^{n_Y} R(Y_i) - [n_Y(n_X + n_Y + 1)/2]}{\sqrt{n_X n_Y (n_X + n_Y + 1)/12}},$

where $R(Y_i)$ denotes the rank of Y_i amongst the combined samples, i.e., amongst $(X_1, \dots, X_{n_X}, Y_1, \dots, Y_{n_Y})$.

Distribution of Z under H_0 : $Z \sim N(0, 1)$.

Implementation 1:

statistic = -4.361334 , p-value = 1.292716e-05

Implementation 2:

W = 284, p-value = 6.15e-07

alternative hypothesis: true location shift is not equal to 0

95 percent confidence interval:

0.89647 1.57023

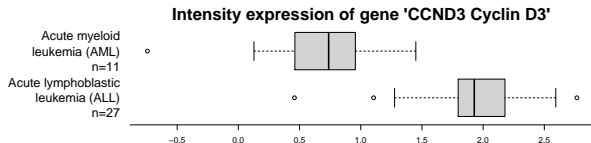
sample estimates:

difference in location

1.21951



F-test of equality of variances

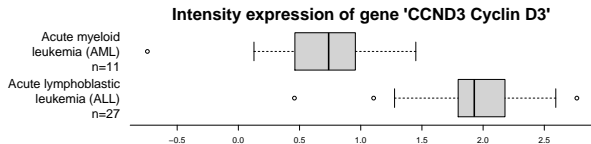


We test $H_0: \sigma_Y^2 = \sigma_X^2$ against $H_1: \sigma_Y^2 \neq \sigma_X^2$.

We know:

► F-test [assume $X_i \sim N(\mu_X, \sigma_X)$ and $Y_i \sim N(\mu_Y, \sigma_Y)$]: $\frac{s_Y^2}{s_X^2} \sim F_{n_Y-1, n_X-1}$

F-test of equality of variances



We test $H_0: \sigma_Y^2 = \sigma_X^2$ against $H_1: \sigma_Y^2 \neq \sigma_X^2$.

We know:

► F-test [assume $X_i \sim N(\mu_X, \sigma_X)$ and $Y_i \sim N(\mu_Y, \sigma_Y)$]: $\frac{s_Y^2}{s_X^2} \sim F_{n_Y-1, n_X-1}$

F test to compare two variances

```
data: golub[1042, gol.fac == "ALL"] and golub[1042, gol.fac == "AML"]
F = 0.71164, num df = 26, denom df = 10, p-value = 0.4652
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
 0.2127735 1.8428387
sample estimates:
ratio of variances
 0.7116441
```

Warning

Multiplicity correction

For each test, the probability of rejecting H_0 (and accept H_1) when H_0 is true equals α .

For k tests, the probability of rejecting H_0 (and accept H_1) at least 1 time when H_0 is true, α_k , is given by

$$\alpha_k = 1 - (1 - \alpha)^k.$$

Thus, for $\alpha = 0.05$,

- ▶ if $k = 1$, $\alpha_1 = 1 - (1 - \alpha)^1 = 0.05$,
- ▶ if $k = 2$, $\alpha_2 = 1 - (1 - \alpha)^2 = 0.0975$,
- ▶ if $k = 10$, $\alpha_{10} = 1 - (1 - \alpha)^{10} = 0.4013$.

Idea: change the level of each test so that $\alpha_k = 0.05$:

- ▶ Bonferroni correction : $\alpha = \frac{\alpha_k}{k}$,
- ▶ Dunn-Sidak correction: $\alpha = 1 - (1 - \alpha_k)^{1/k}$.

Warning

Non-parametric is not assumption free: Type I error

Simulate 2500 samples with

- ▶ $X_i \sim \text{Uniform}(1.5, 2.5)$, $i = 1, \dots, n_X$,
- ▶ $Y_i \sim \text{Uniform}(0, 4)$, $i = 1, \dots, n_Y$,

so that $E[X_i] = E[Y_i] = 2$ (i.e., same mean, same median).

Assume

- ▶ $X_i \sim \text{iid}(\mu_X, \sigma^2)$, $i = 1, \dots, n_X$,
- ▶ $Y_i \sim \text{iid}(\mu_X + \delta, \sigma^2)$, $i = 1, \dots, n_Y$.

Test **H0**: $\delta = \delta_0$ against **H1**: $\delta \neq \delta_0$, at the 5% level, by means of

- ▶ Mann-Whitney-Wilcoxon test (MWW),
- ▶ T-test,
- ▶ Welch-test.

$\hat{\alpha}$		Tests		
Sample size		MWW	Student's t-test	Welch's test
	$n_X = 200, n_Y = 70$	0.145	0.202	0.055
	$n_X = 20, n_Y = 7$	0.148	0.240	0.062

Exercises