Statistics primer: Hypothesis testing

Laurent Gatto

January 21, 2016

Foreword

> *the function of significance tests is to prevent you from making a fool of yourself, and not to make unpublishable results publishable [1]*

at the end of the course, we will understand

> *Why Most Published Research Findings Are False. [3]*

and will pledge to not make (too frequently) fools of ourselves.

Statistical hypothesis testing

- Testing a working hypothesis
- Contrast an observed result to a *comparable* random distribution (assumptions!). If it is *different enough* from what we would expect by chance, then we might have an interesting result.

The testing process

1. Set up a model of reality: null hypothesis H_0 (no difference with random distribution; as opposed to *alternative hypothesis* H_1, there is a difference with random distribution)
2. Do an experiment, collect data
3. Compute the probability of the data in this model
4. Make a decision: reject H_0 if the computed probability is deemed too small.

Choosing a test statistic

- Continuous data (such as micro-array, quantitative proteomics, . . .) and we want to compare means: *t-test*
- Count data (high-throughput sequencing) and we want to compare the number of reads between two conditions: *negative binomial*
- Gene set enrichment (is there an enrichment of genes with a specific function in my set of interesting genes): *hypergeometric*

Check what is used in the literature. Use state-of-the-art methods/software that have been specifically developed for the desing/technology at hand.

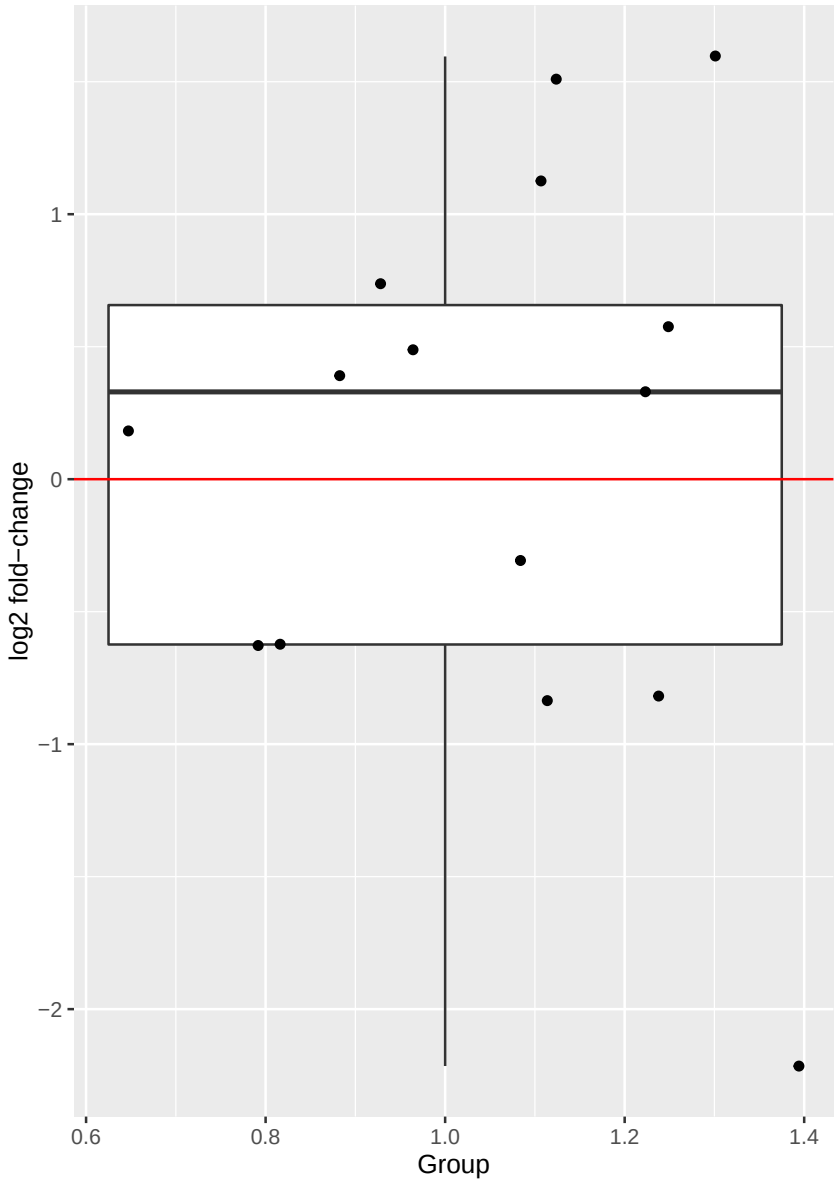
Type of tests: (non-)parametric

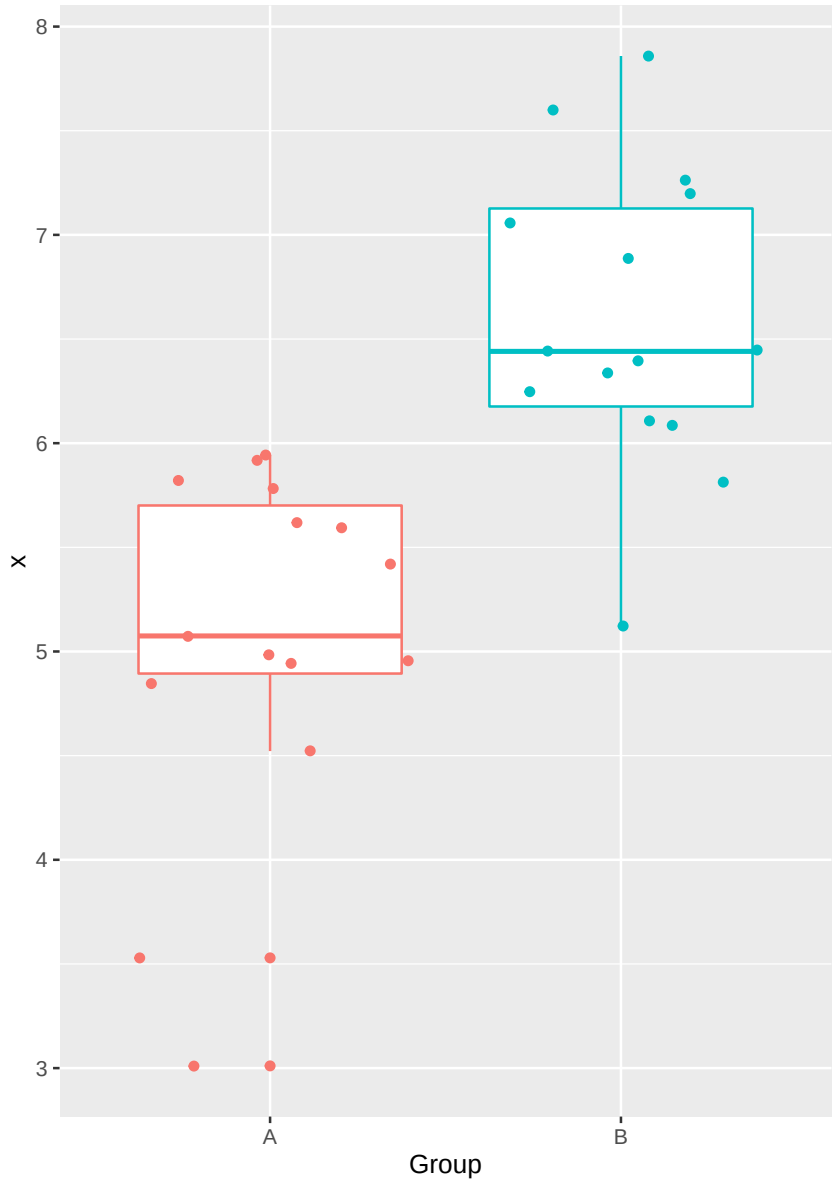
- Parametric: assumption is that the data comes from a population that follows a probability distribution
- Non-parametric: no defined/fixed parameters

When assumptions are correct, a parametric test has more *statistical power*.

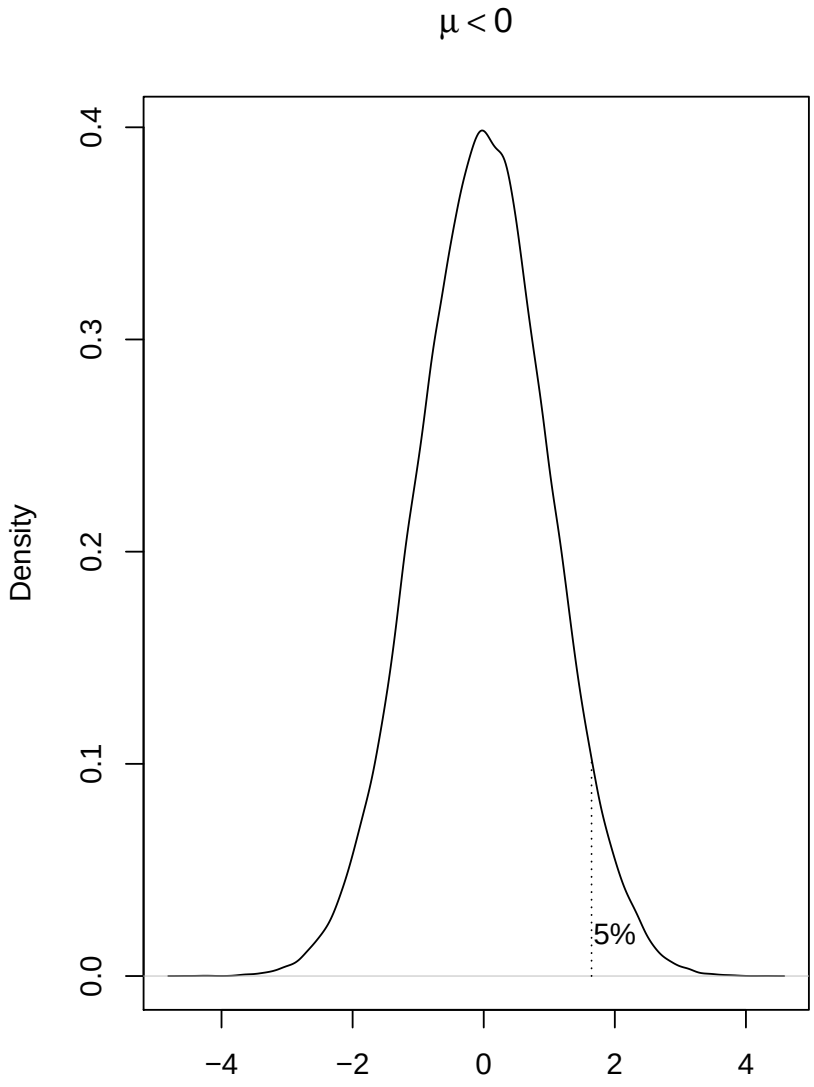
In practice, as most studies are under-powered, non-parametric tests are not an option.

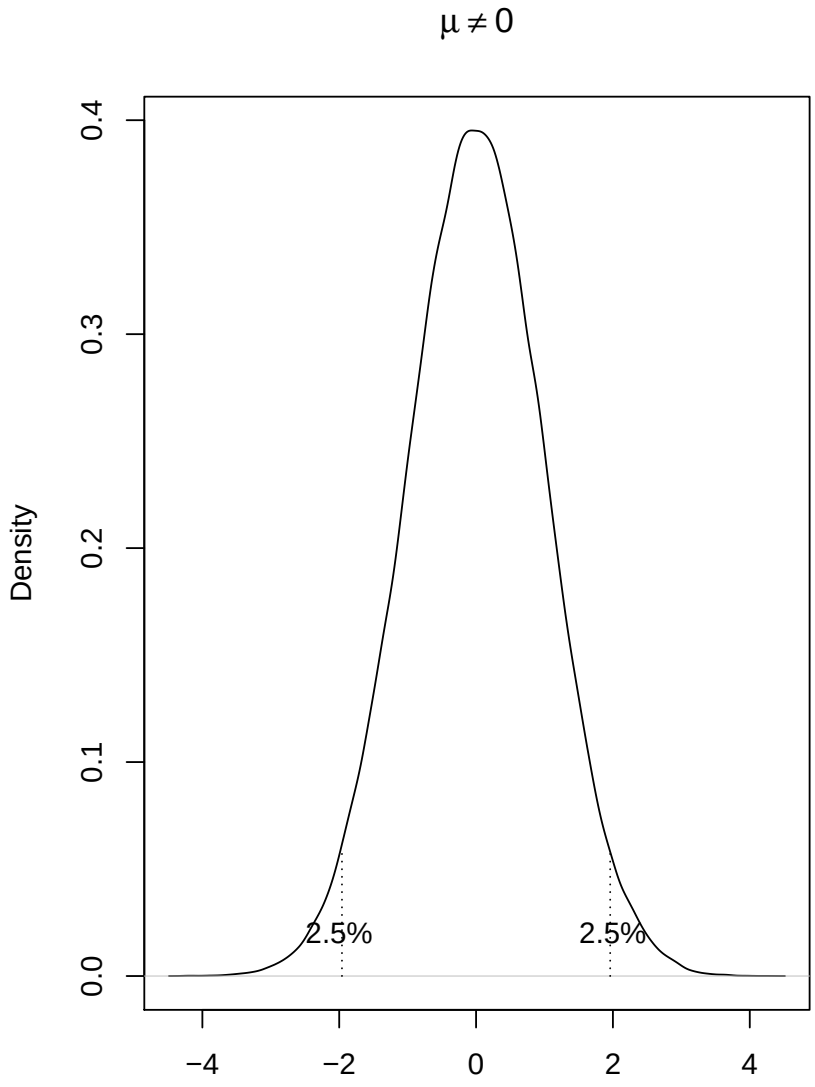
Types of tests: One-sample or two-sample tests





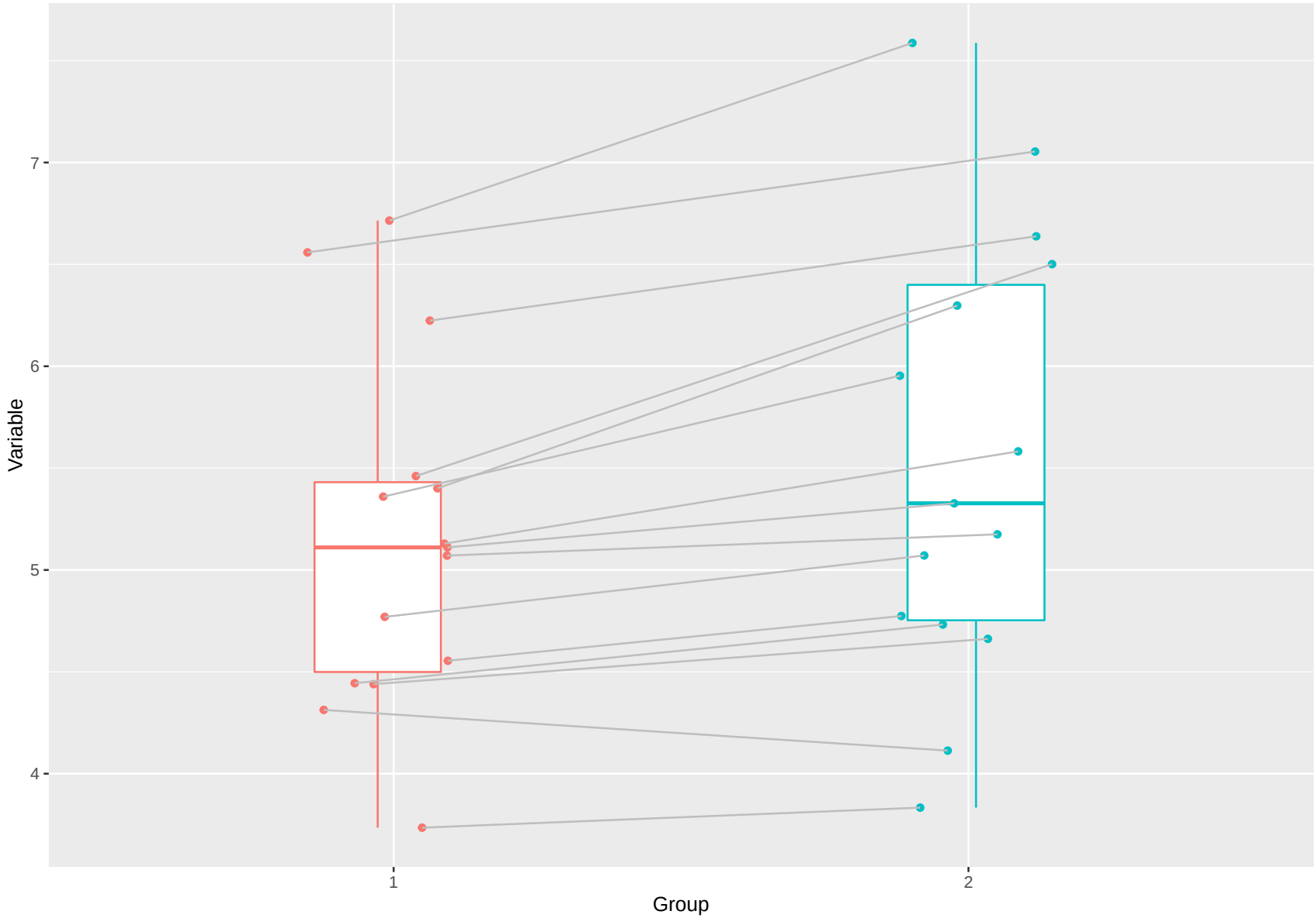
One-sides vs two-sided tests





Types of tests: paired tests

Measurements in two conditions are paired.



Working example: Student's t-test

Comparing means from 2 groups, continuous data.

- $H_0 : \mu_1 = \mu_2$
- $H_1 : \mu_1 \neq \mu_2$

Assumptions:

- data is normally distributed
- data are independent and identically distributed
- equal or un-equal (Welch test) variance
- t-test is robust to deviations.

(All models are wrong. Some are useful)

Welch test (t-test with unequal variances)

Two samples of sizes n_1 and n_2

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_{\bar{x_1} - \bar{x_2}}}$$

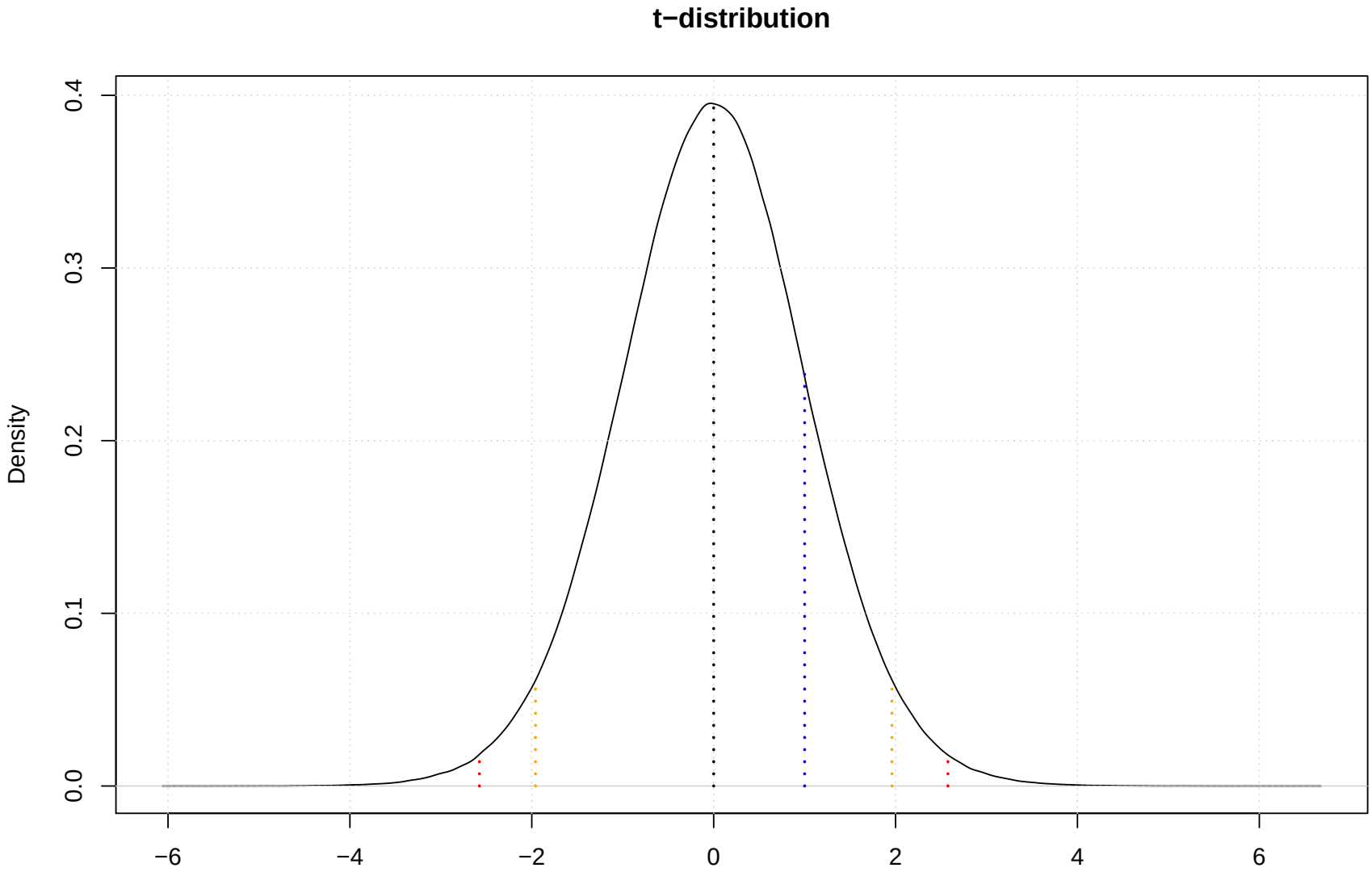
where

$$s_{\bar{x_1} - \bar{x_2}} = \sqrt{\frac{s_{x_1}^2}{n_1} + \frac{s_{x_2}^2}{n_2}}$$

and s_{xi} is the standard deviation of sample i

$$s_{x_i} = \sqrt{\frac{1}{N-1} \sum_{i=1}^{N} (x_i - \bar{x})^2}$$

How to we estimate how different we are from *random*



p-value: how (un)likely it would be to observe a value as extreme or more extreme under H_0.

P-value

p-value: how (un)likely it would be to observe a value as extreme or more extreme under H_0.

If p-value $<=$ than (**arbitrary**) significance level, then we reject H_0.

Avoid fallacy

The p-value is the probability that the data could happen, under the condition that the null hypothesis is true.

It is not the probability that the null hypothesis is true.

Absence of evidence $\neq$ evidence of absence

What can go wrong

	H_0 is true	H_0 is false
H_0 is rejected	Type I error, FP	correct TP
H_0 not rejected	correct TN	Type II error, FN

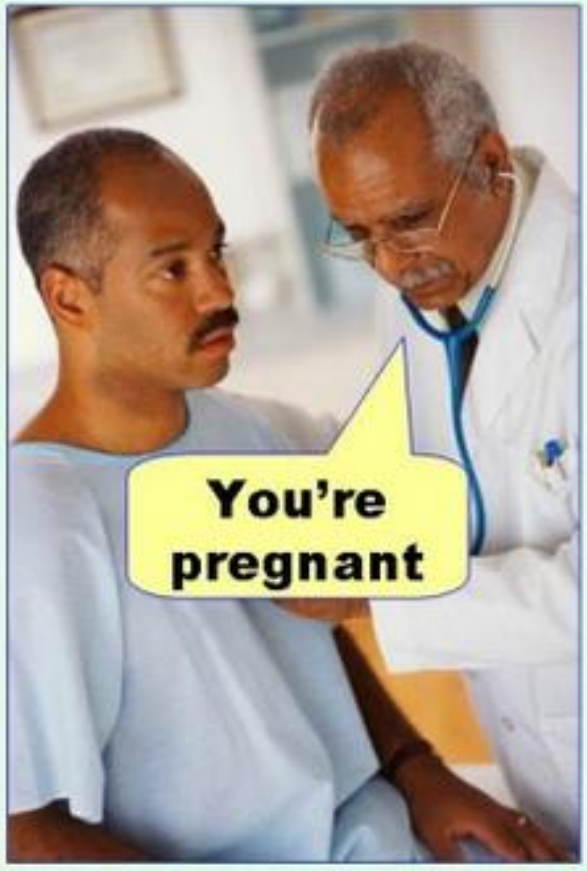
Type I and II errors are not independent. One can't control both.

power of a test $= 1$ - *type II*

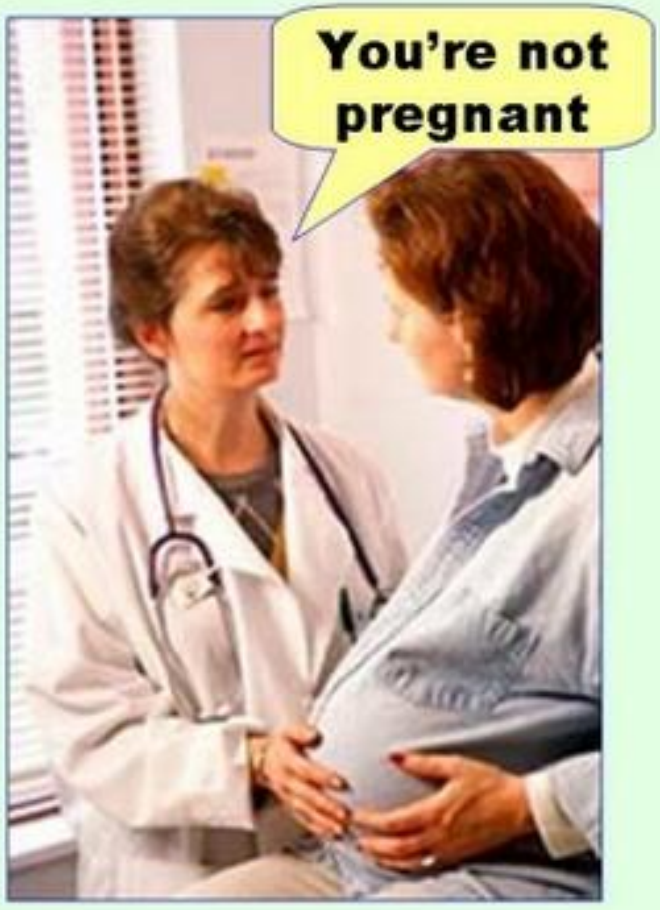
(FP: false positive, TP: true positive, FN: false negative, TN: true positive)

False discovery rate: $\frac{FP}{FP+TP}$

Type I error
(false positive)



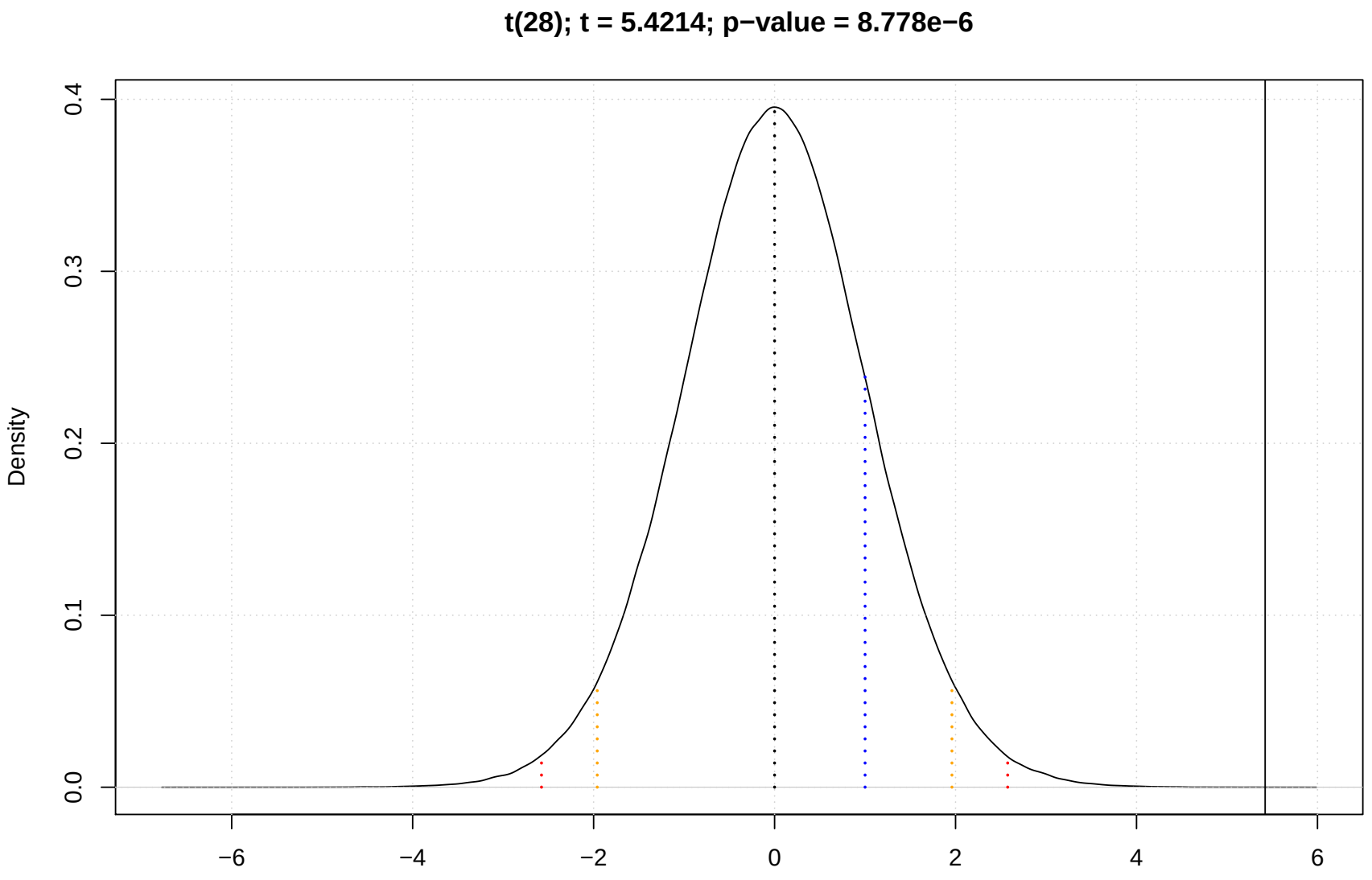
Type II error
(false negative)



In R

```r
x <- rnorm(15, mean = 7, sd = 1)
y <- rnorm(15, mean = 5, sd = 1.2)
t.test(x, y)
```

```
##
##  Welch Two Sample t-test
##
## data:  x and y
## t = 5.4214, df = 27.998, p-value = 8.778e-06
## alternative hypothesis: true difference in means is not
## 95 percent confidence interval:
##  1.259224 2.788685
## sample estimates:
## mean of x mean of y
##  7.100843  5.076888
```

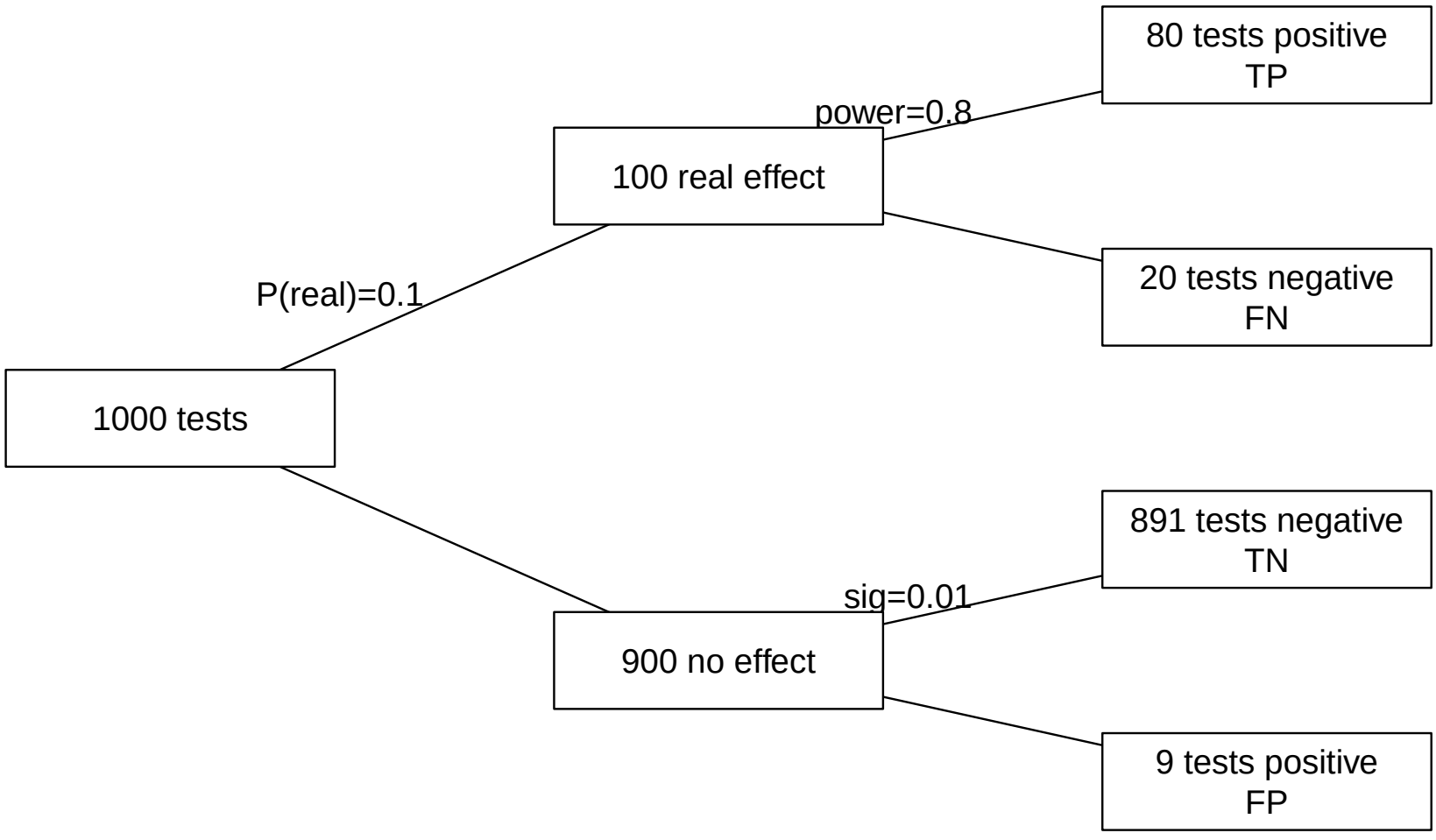

t(28); t = 5.4214; p−value = 8.778e−6
Density
0.0
0.1
0.2
0.3
0.4
−6
−4
−2
0
2
4
6

But

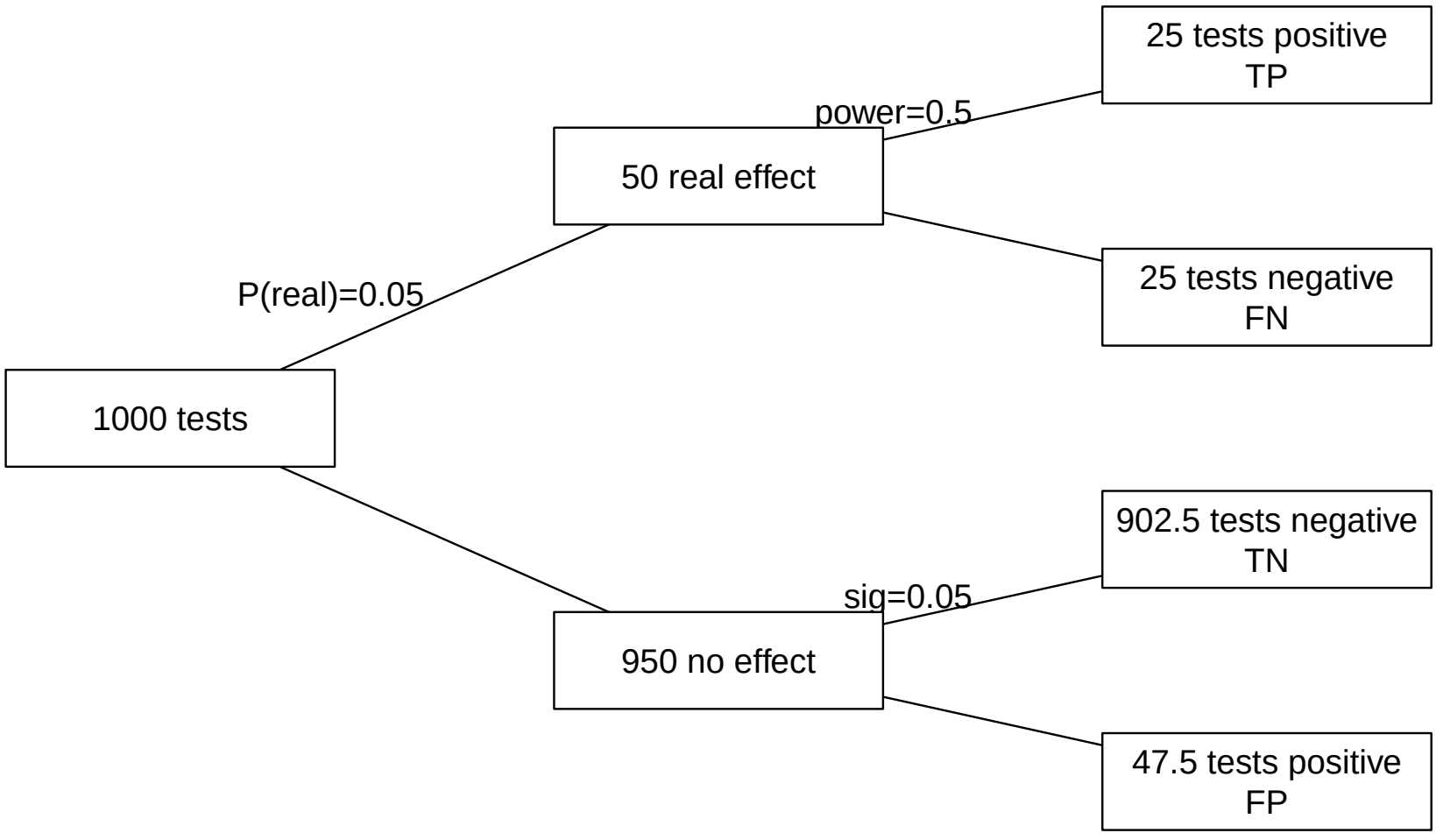
Is the p-value really what we want? What is the probability that we make a fool of ourselves?

What about the power of our test? Let's calculate a false positive rate.

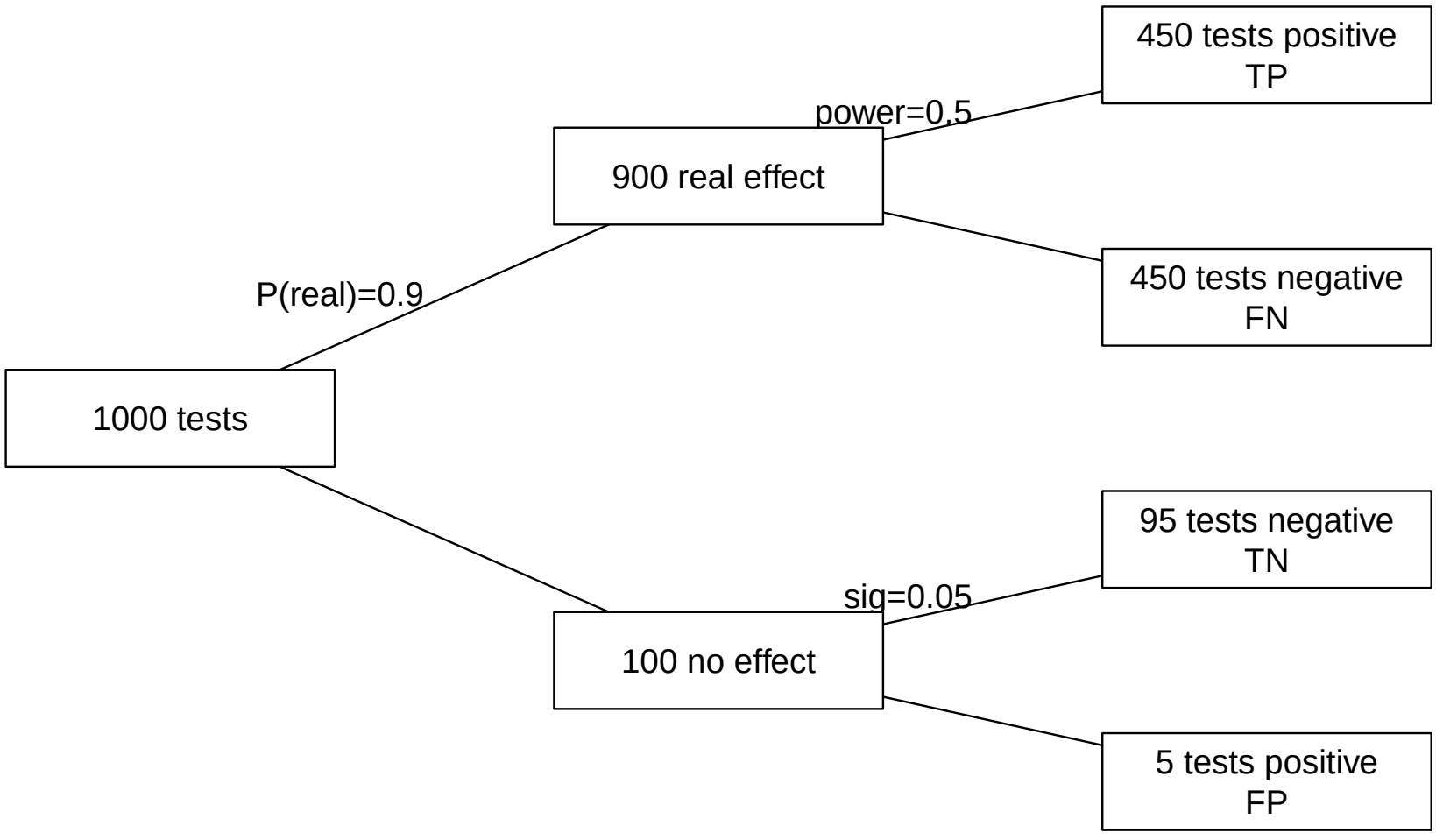
False discovery rate: 9/(80+9) = 0.101



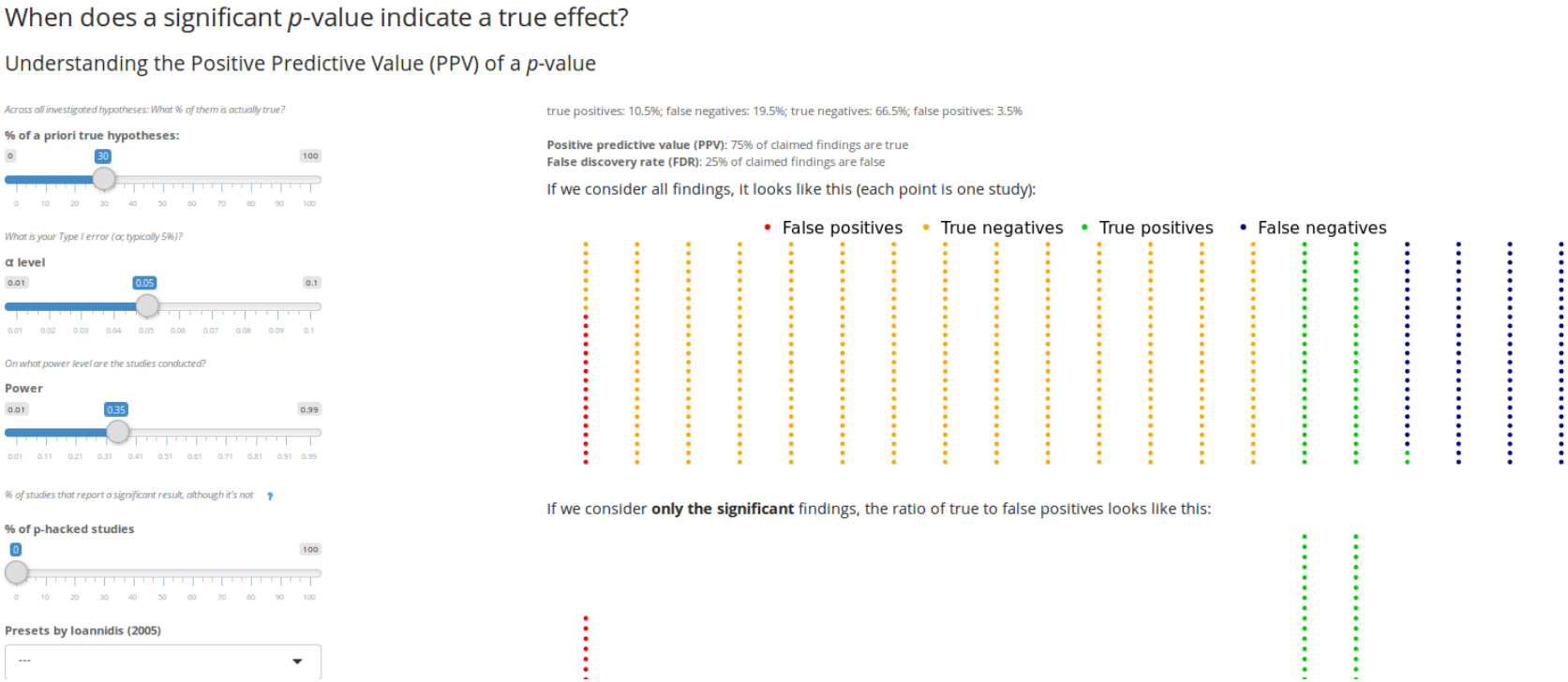
False discovery rate: 47.5/(25+47.5) = 0.655

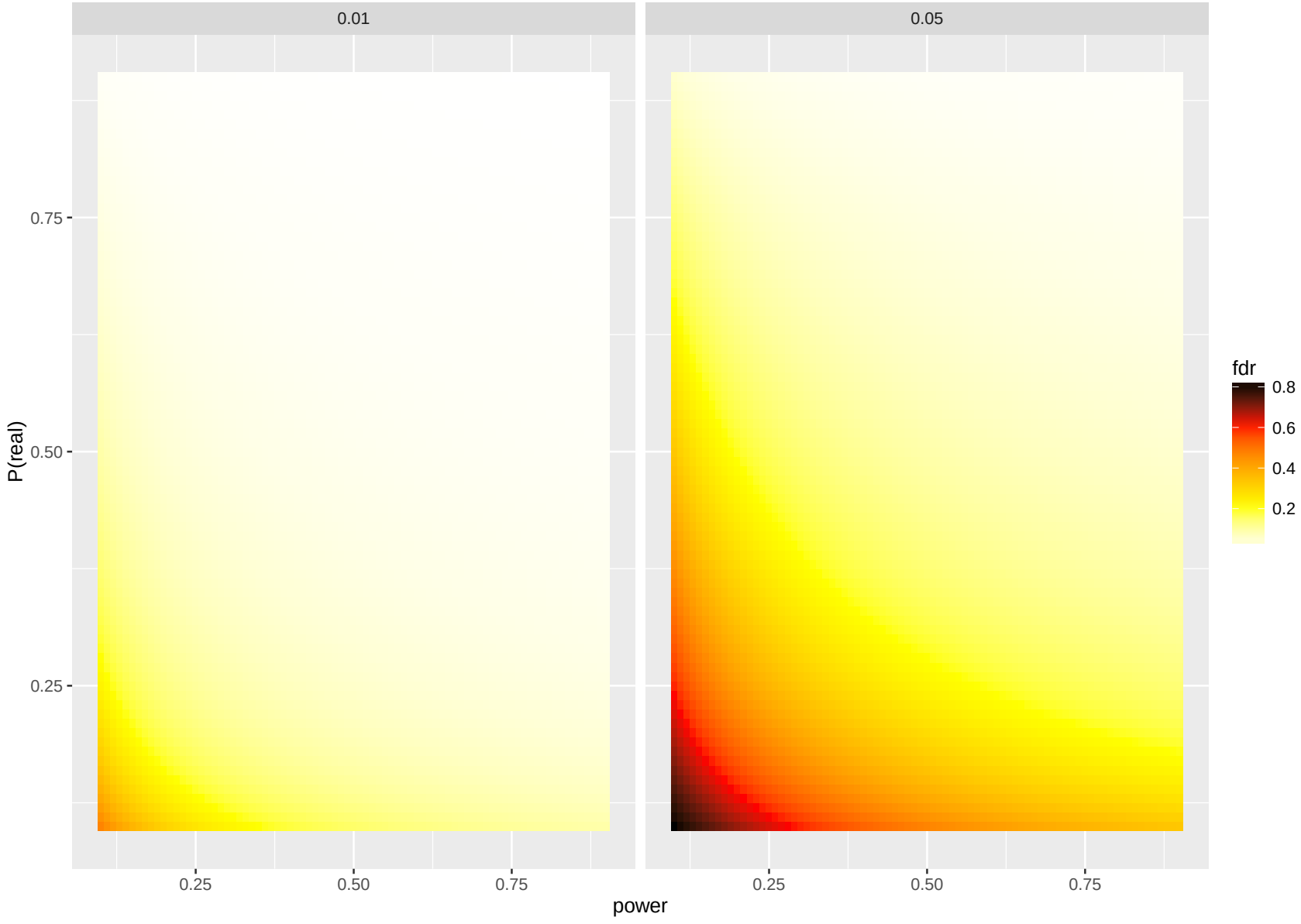


False discovery rate: 5/(450+5) = 0.011



See also this interactive app `http://shinyapps.org/apps/PPV/`.

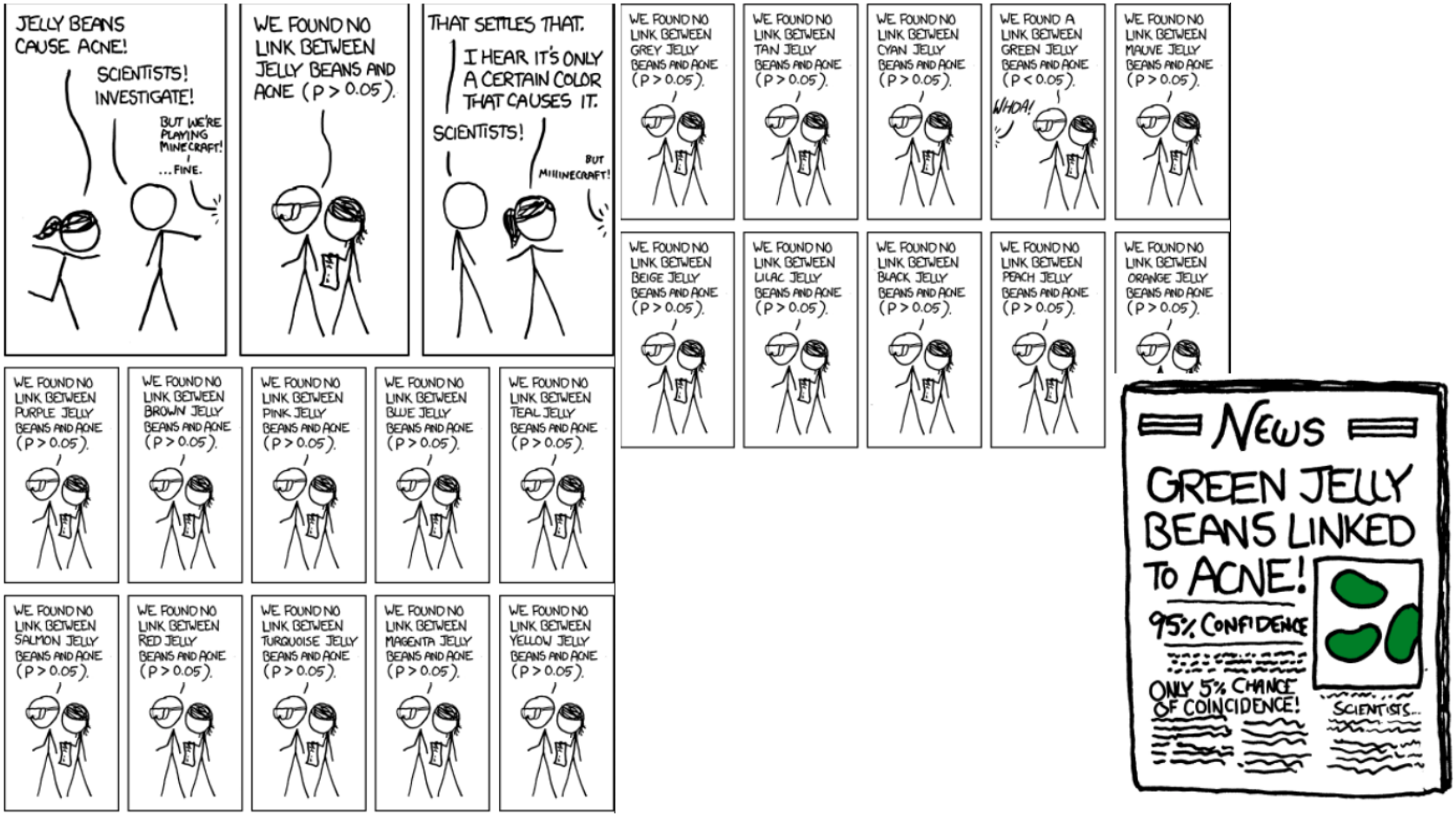



0.01
0.05
P(real)
0.75
0.50
0.25
0.25
0.50
0.75
0.25
0.50
0.75
power
fdr
0.8
0.6
0.4
0.2

Adjustment for multiple testing

A p-value informs us about a single test. What if we have 1000s or more tests?

Jelly beans can cause (xkcd)



Adjustment for multiple testing

A p-value informs us about a single test. What if we have 1000s or more tests?

As the number of comparisons increases, it becomes more likely that the some results will appear to differ by chance. Our confidence that a result will generalise to independent data should generally be weaker if it is observed as part of an analysis that involves multiple comparisons, rather than an analysis that involves only a single comparison.

For example, if one test is performed at the 5% level, there is only a 5% chance of incorrectly rejecting the null hypothesis if the null hypothesis is true. However, for 100 tests where all null hypotheses are true, the expected number of incorrect rejections is 5. These errors are **false positives**.

Let's try it (1)

```
t.test(rnorm(5), rnorm(5))
```

```
##
##  Welch Two Sample t-test
##
## data:  rnorm(5) and rnorm(5)
## t = -0.011202, df = 7.1385, p-value = 0.9914
## alternative hypothesis: true difference in means is not
## 95 percent confidence interval:
##  -1.239198  1.227467
## sample estimates:
## mean of x mean of y
## 0.1292699 0.1351357
```

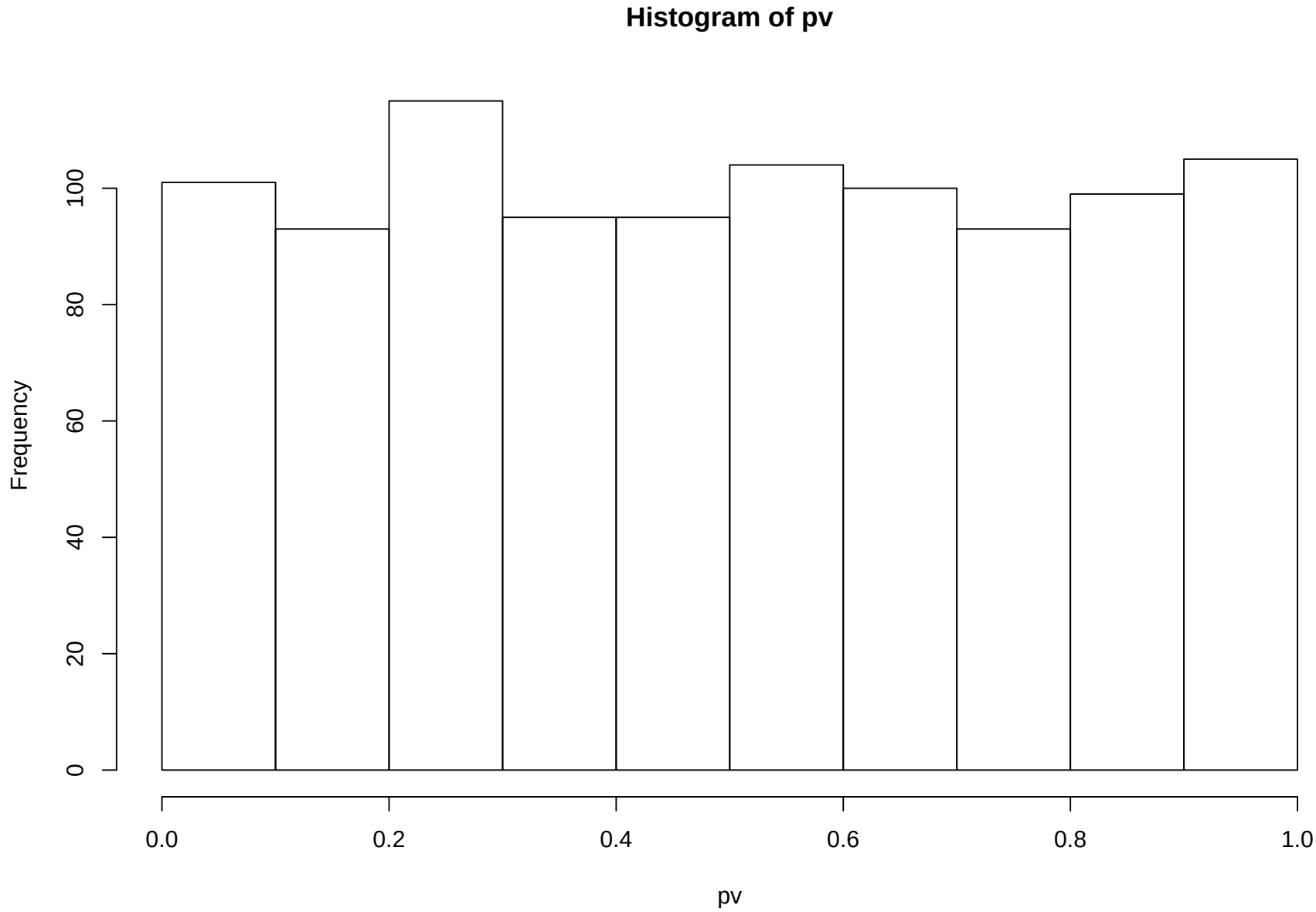
Let's try it (1)

```
pv <- replicate(1000, t.test(rnorm(5), rnorm(5))$p.value)
head(sort(pv))
```

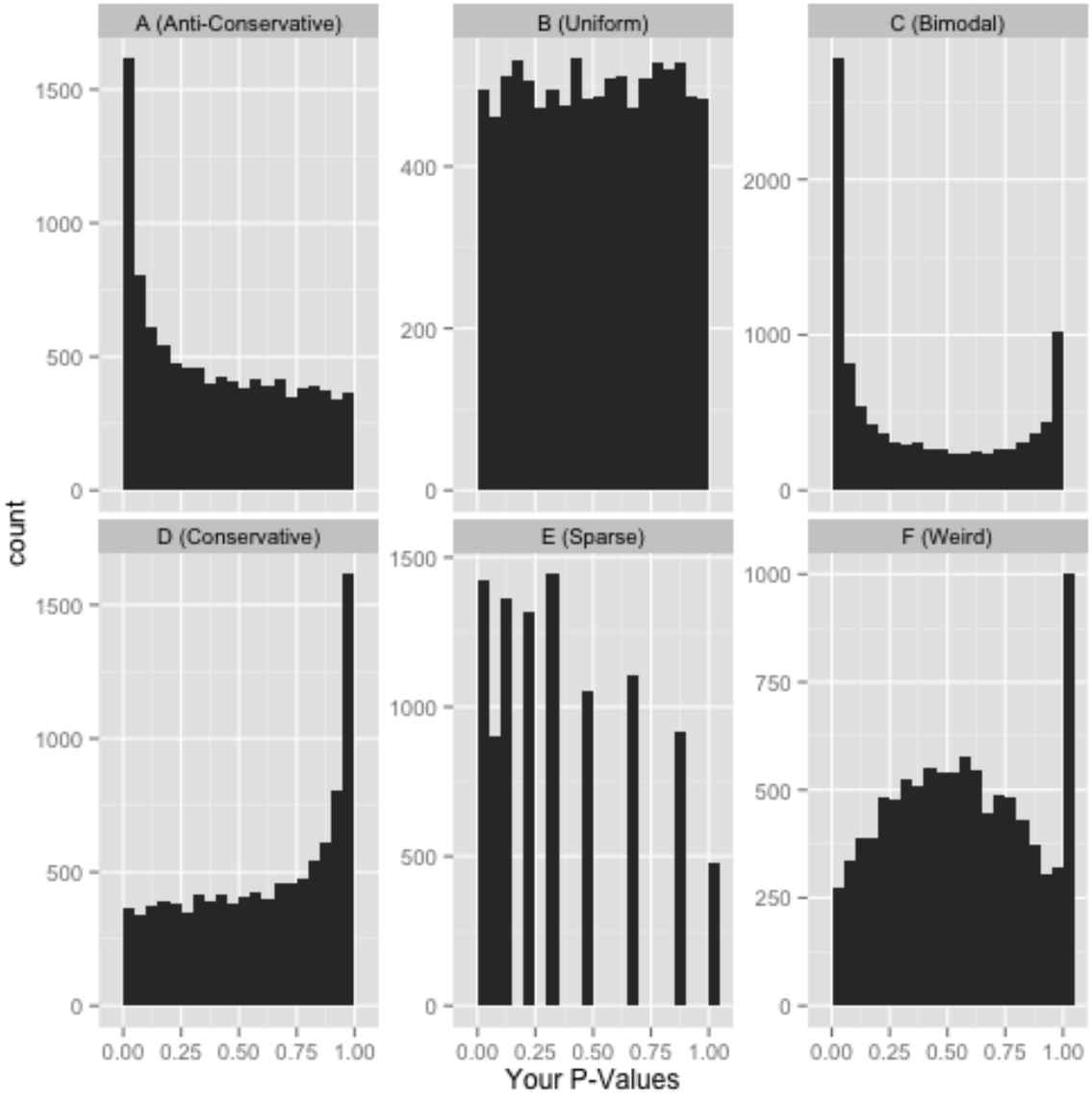
```
## [1] 0.001912827 0.002889056 0.002938096 0.003023485 0.00
```

```
table(pv < 0.05)
```

```
##
## FALSE  TRUE
##   956    44
```


Histogram of pv
Frequency
0
20
40
60
80
100
0.0
0.2
0.4
0.6
0.8
1.0
pv

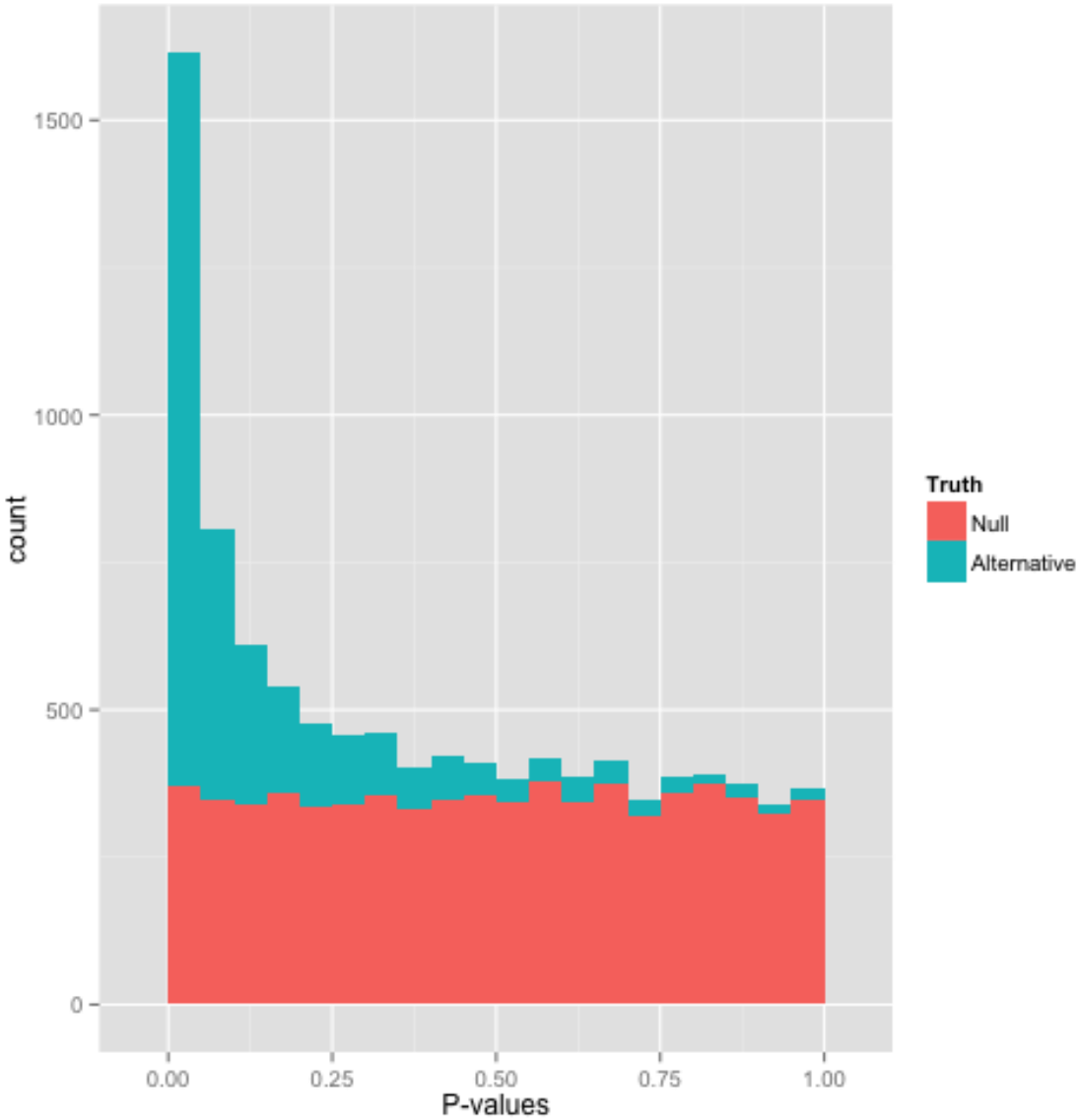
How to interpret a p-value histogram (link)



Adjusting for multiple comparisons

- **Family-wise error rate** (FWER) The probability of one or more false positives. **Bonferroni correction** For m tests, multiply each p-value with m. Then see if anyone still remains below significance threshold.
- **False discovery rate** (FDR): The expected fraction of false positives among all discoveries. Allows us to choose n results with a given FDR. Examples are Benjamini-Hochberg or q-values.

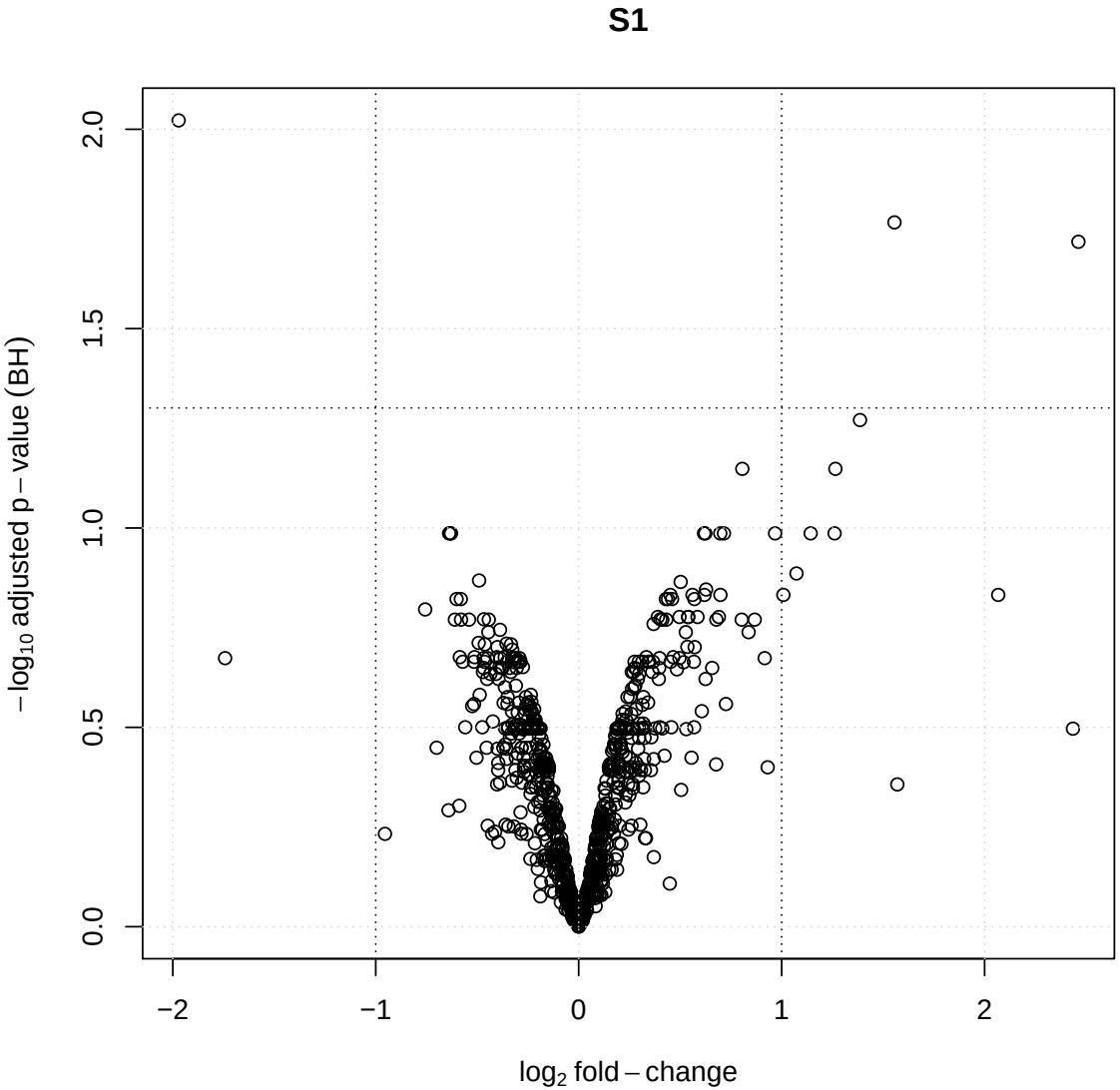
FDR adjustment



Observed p-values are a mix of samples from a uniform (**nulls**) and anti-conservative (**alternatives**) distributions.

Identifying positive candidates

Volcano plots: (adjusted) p-values vs effect size (fold-change)



Why are most published research findings false

- fishing for $p < 0.05$ (p-hacking)
- under-powered studies
- poor data analysis practice

Also

- Omitting null results
- Under-specified methods
- Weak experimental design

(see also the **Reproducibility and the conduct of research**)

What can we do

- Beware of **arbitrary thresholds**, look for **outliers**: does changing one parameter suddenly change your biological results substantially?
- The choice of level of significance should be based on the relative consequences of the 2 types of errors.
- Consider **p-value and effect** (for example volcano plot).
- Look at **confidence intervals**.
- Beware of **under-powered** studies.
- Read up what is best in your field (there is some great work out there) - for example the `limma`, `edgeR`, `DESeq2` Bioconductor packages.
- Even if we don't see any effect, doesn't mean there is none. Significance testing is not a way to make unpublishable results publishable.

References

[1] David Colquhoun *An investigation of the false discovery rate and the misinterpretation of p-values* R. Soc. open sci. 2014 1 140216; doi:10.1098/rsos.140216.

[2] Regina Nuzzo *Scientific method: Statistical errors* 2014 Nature 506, 150–152 doi:10.1038/506150a

[3] Ioannidis JPA Why Most Published Research Findings Are False. 2015 PLoS Med 2(8): e124. doi:10.1371/journal.pmed.0020124.

[4] Statistics for biologists web collection.