Introduction to differential gene expression, multiple testing and FDR

Zohar Yakhini, Leon Anavy,
Ben Galili – Reichman.
Partially based on slides from Zohar Yakhini,
Doron Lipson, Itai Sharon and others,
at the Technion

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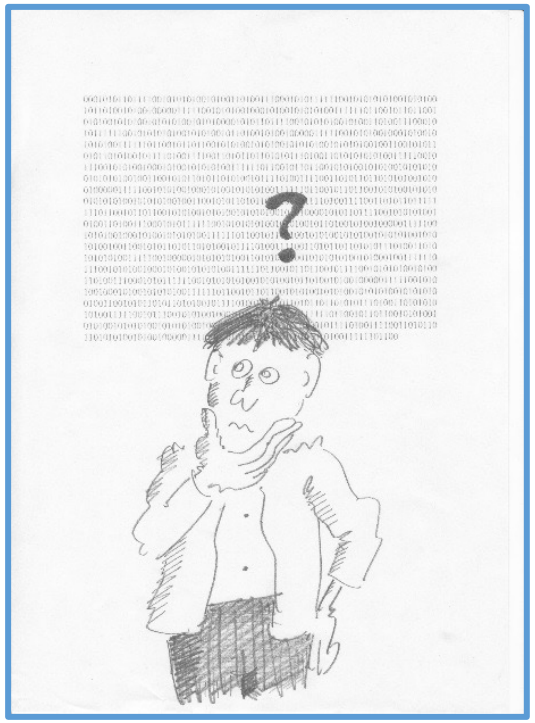

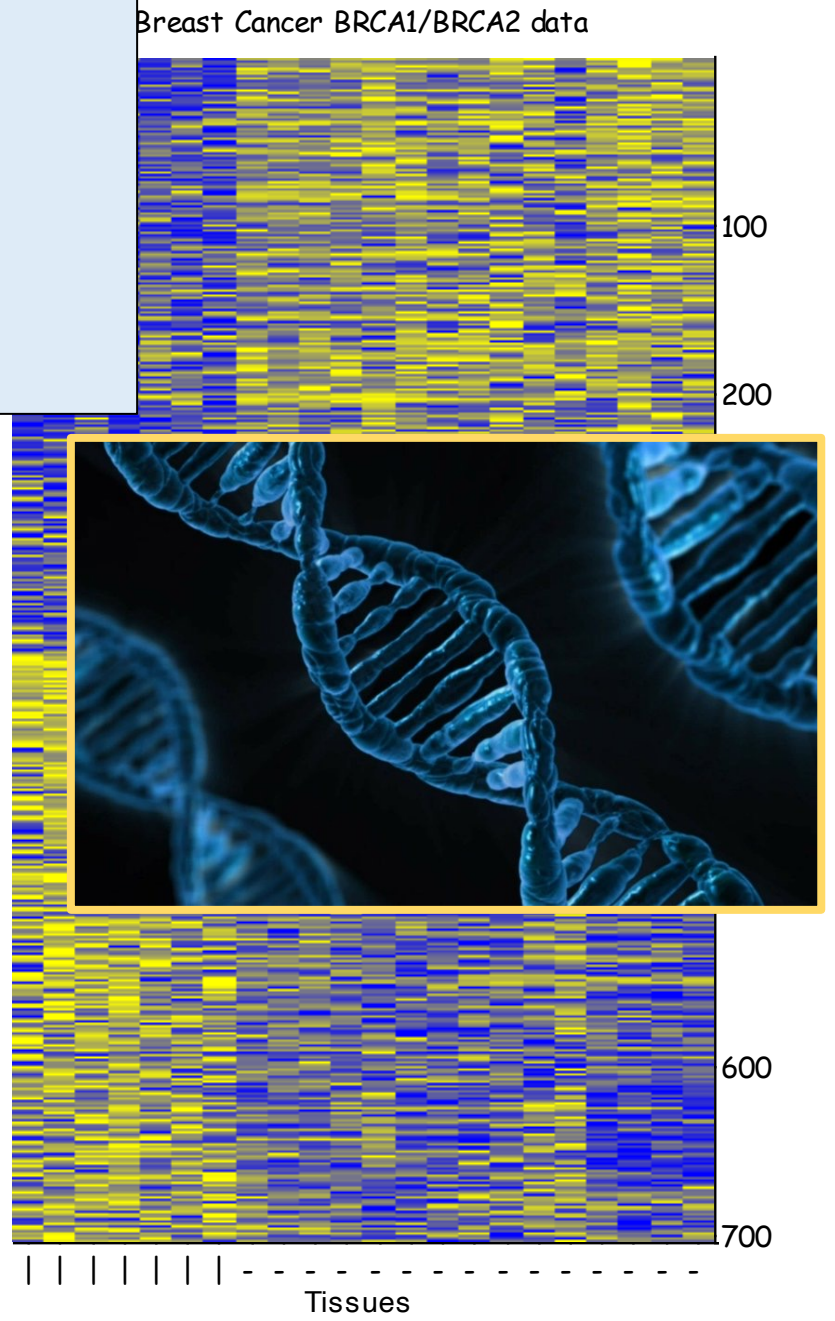

The concept of p-value

Under a given NULL MODEL, what is the probability of observing a value for a MEASURED QUANTITY which is as or more extreme than the one actually measured in data?

We tossed a coin 100 times and observed 27 Hs.

Under a FAIR INDEPENDENT COIN TOSSING MODEL, what is the probability of observing a NUMBER OF Hs which is less than 27 out of 100.

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Tall people

- What is the probability that the person sitting next to you on the bus going back home is >1.90m tall?
- What is the probability of SOME person in the bus being >1.90m tall?
- What is the probability that someone on the IDC campus is >1.90m tall?

$$P(X > 1.9) = 0.01$$

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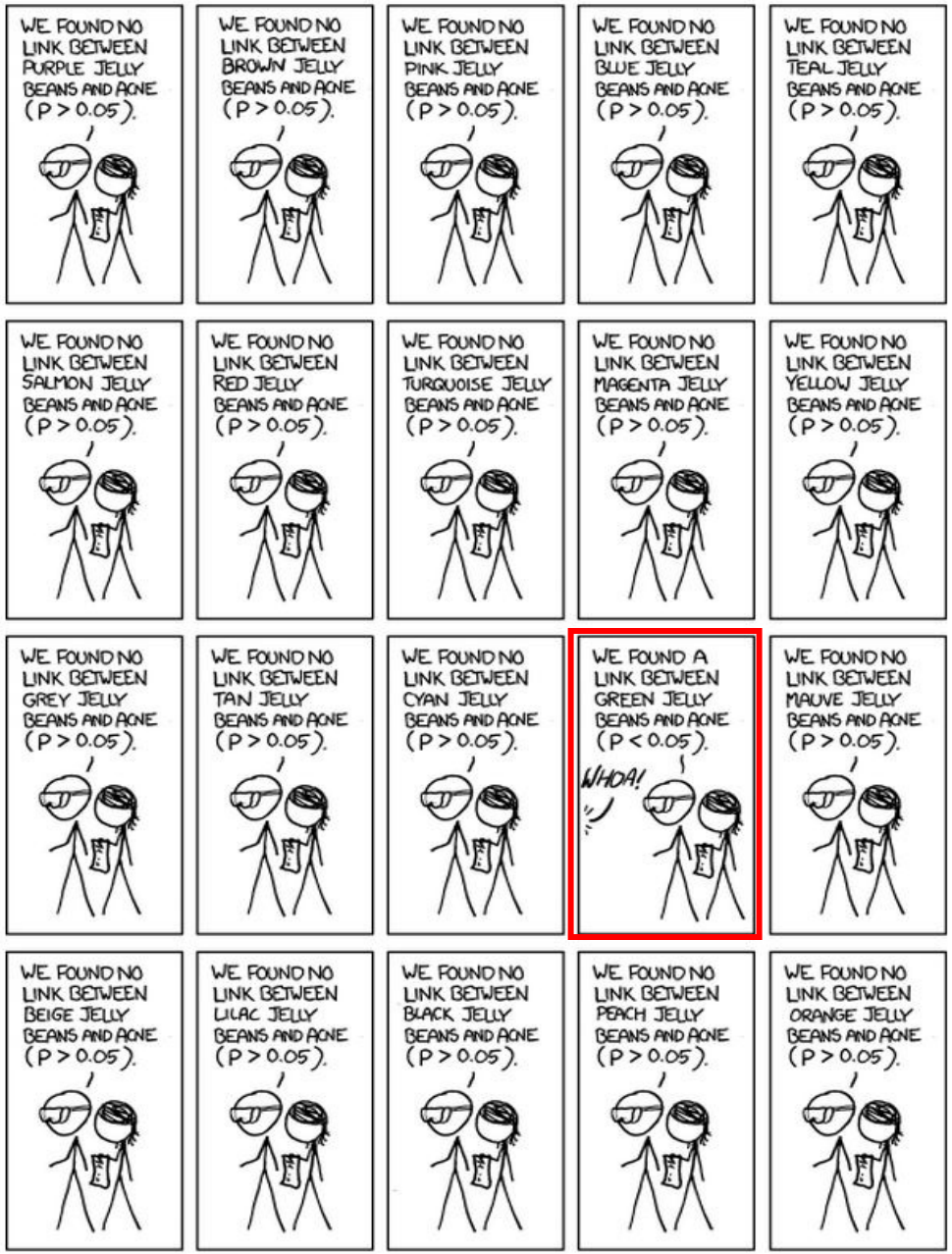
Multiple testing



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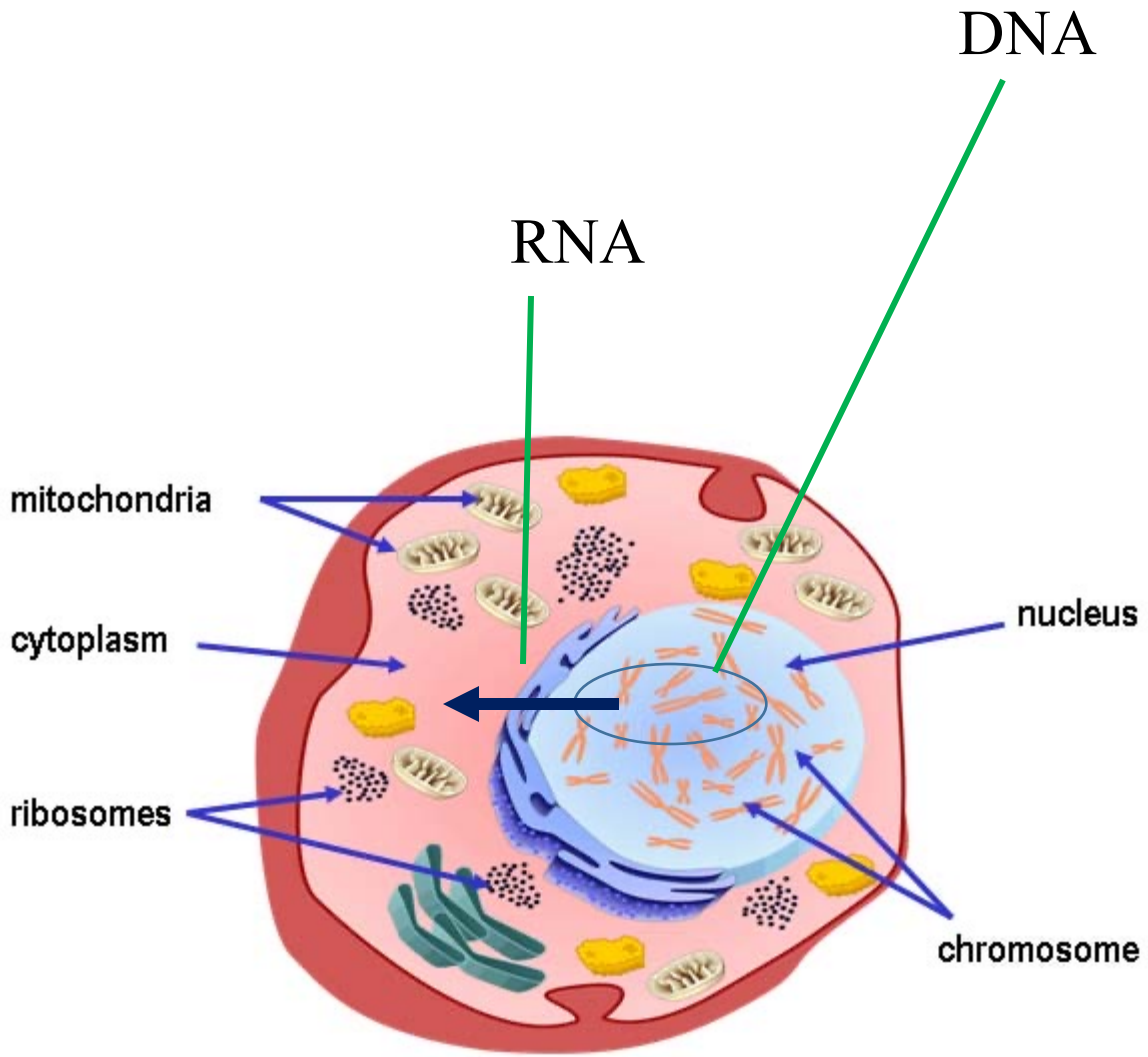
A living cell



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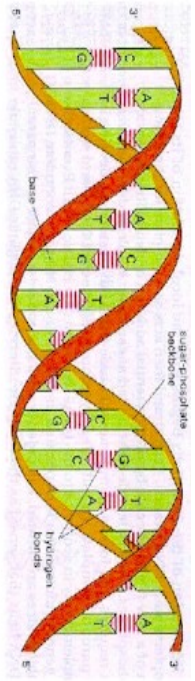
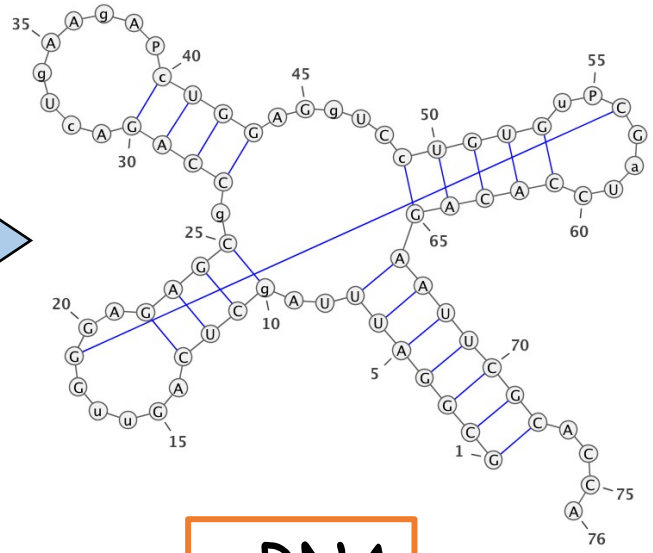
Central Dogma, information flow in living cells



Gene
(DNA, nuclear)

Transcription



mRNA

Translation

Protein

Cells express **different** subsets of the (organismal) genes in different tissues and under different conditions

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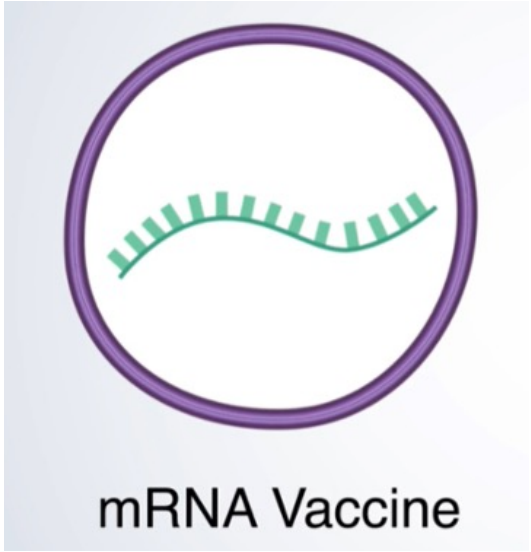
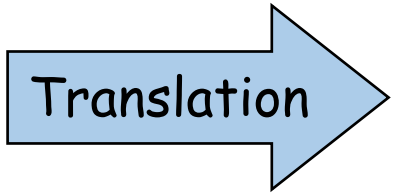
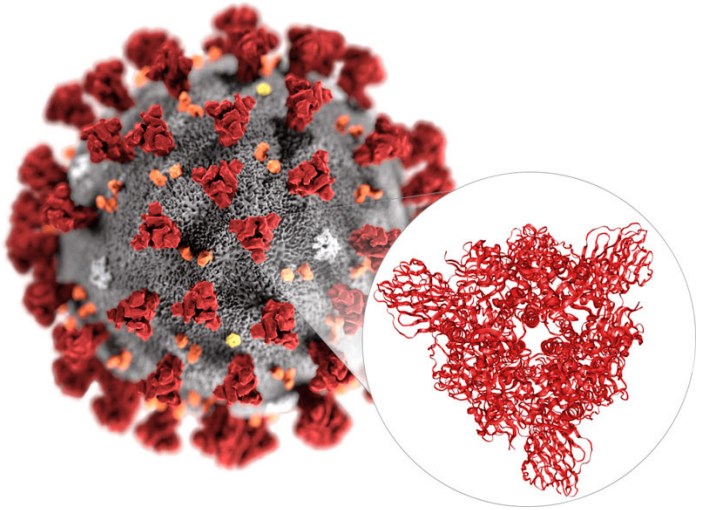
Pfizer COVID-19 mRNA vaccine







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BioNTech CoVid19 vaccine is a 4,284nt mRNA which mimics coding of the pathogen's spike protein.
The entire virus is coded by ~30Knt RNA genome

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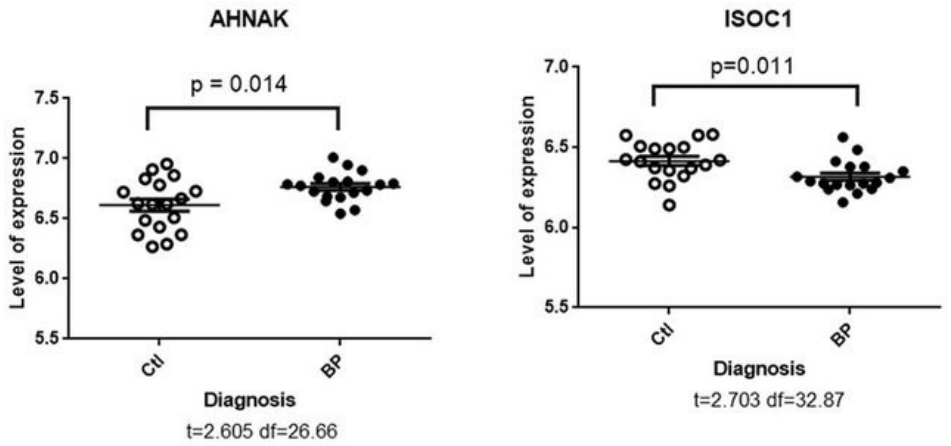
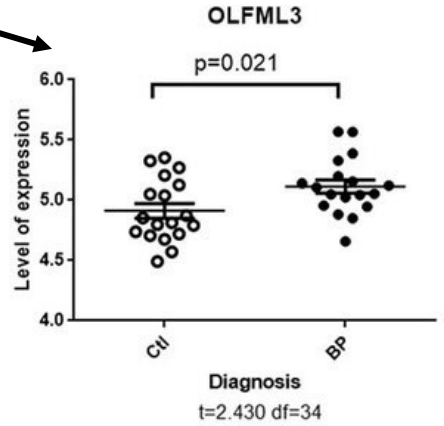
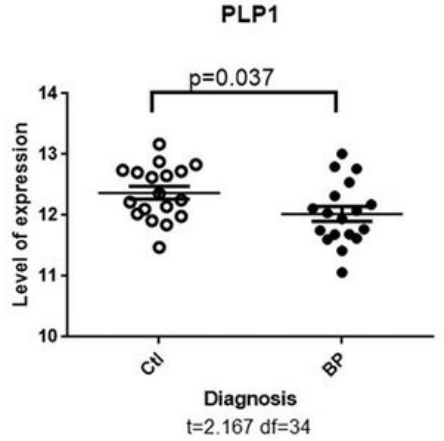
Differentially expressed genes

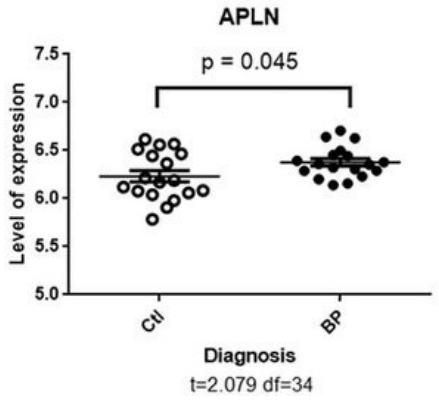
Postmortem measurement of gene expression in brain cells, Bi-Polar (BP) vs Control (Ctl)

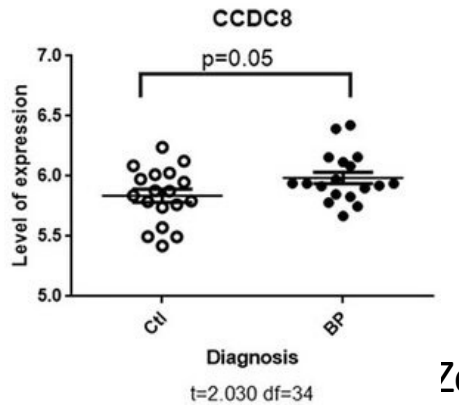
PCR measurement











From:
Kidnapillai et al,
The use of a gene expression signature and connectivity map to repurpose drugs for bipolar disorder,
Ken Walder's Lab,
Biological Psychiatry 2018

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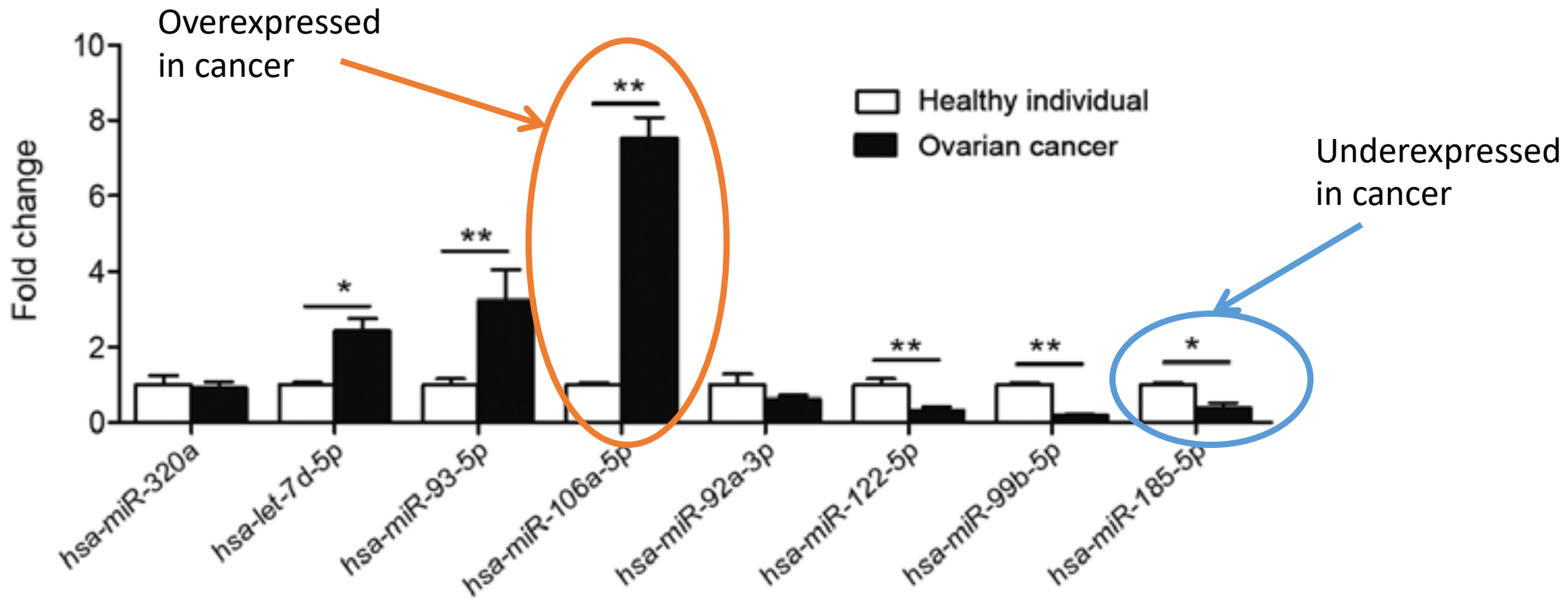
Differentially expressed genes



Expression levels of plasma exosomal miRNA measured in patients with ovarian cancer (n=30) and healthy women (n=30) using RT-qPCR.
*P<0.05 and **P<0.01.

Zhang et al,
Oncology Letters 2019

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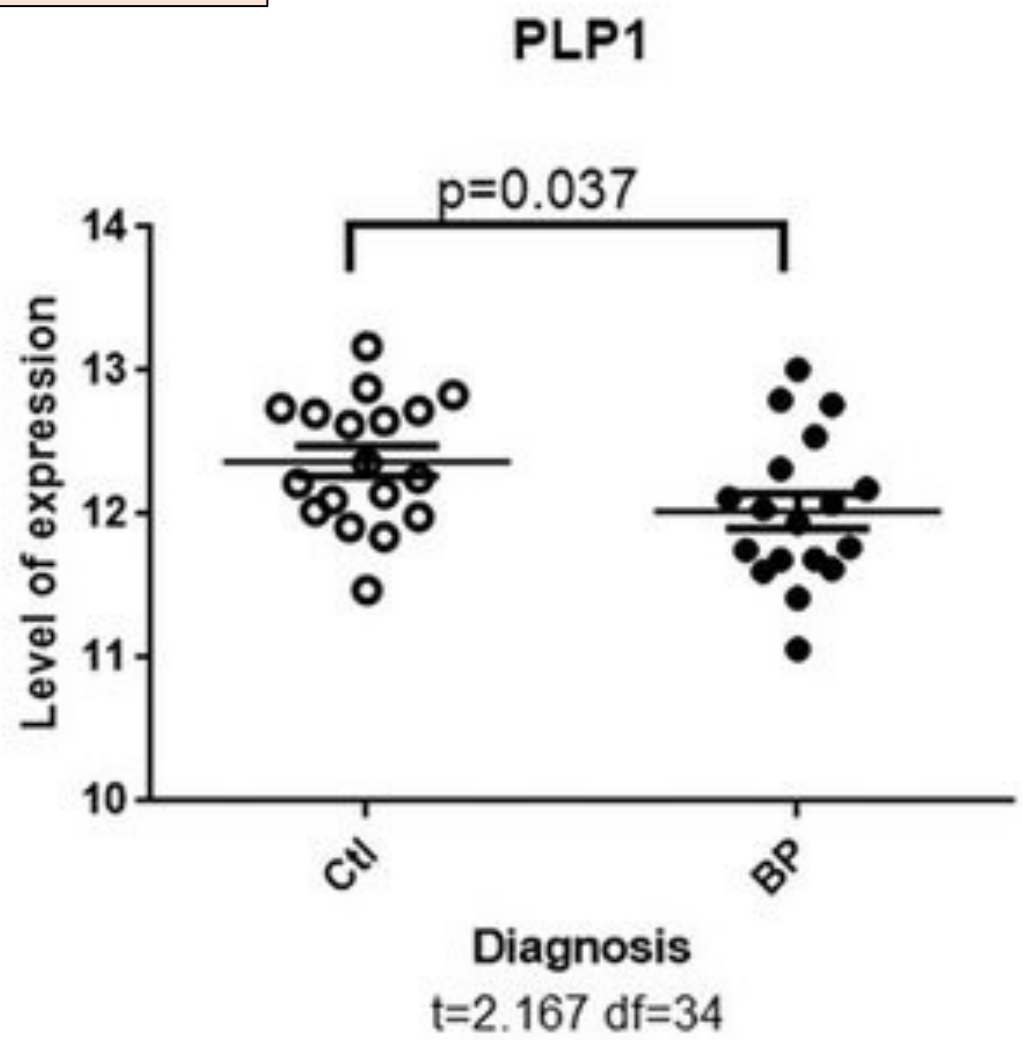
Differentially expressed genes

- t-Test
- WRS
- Other methods…



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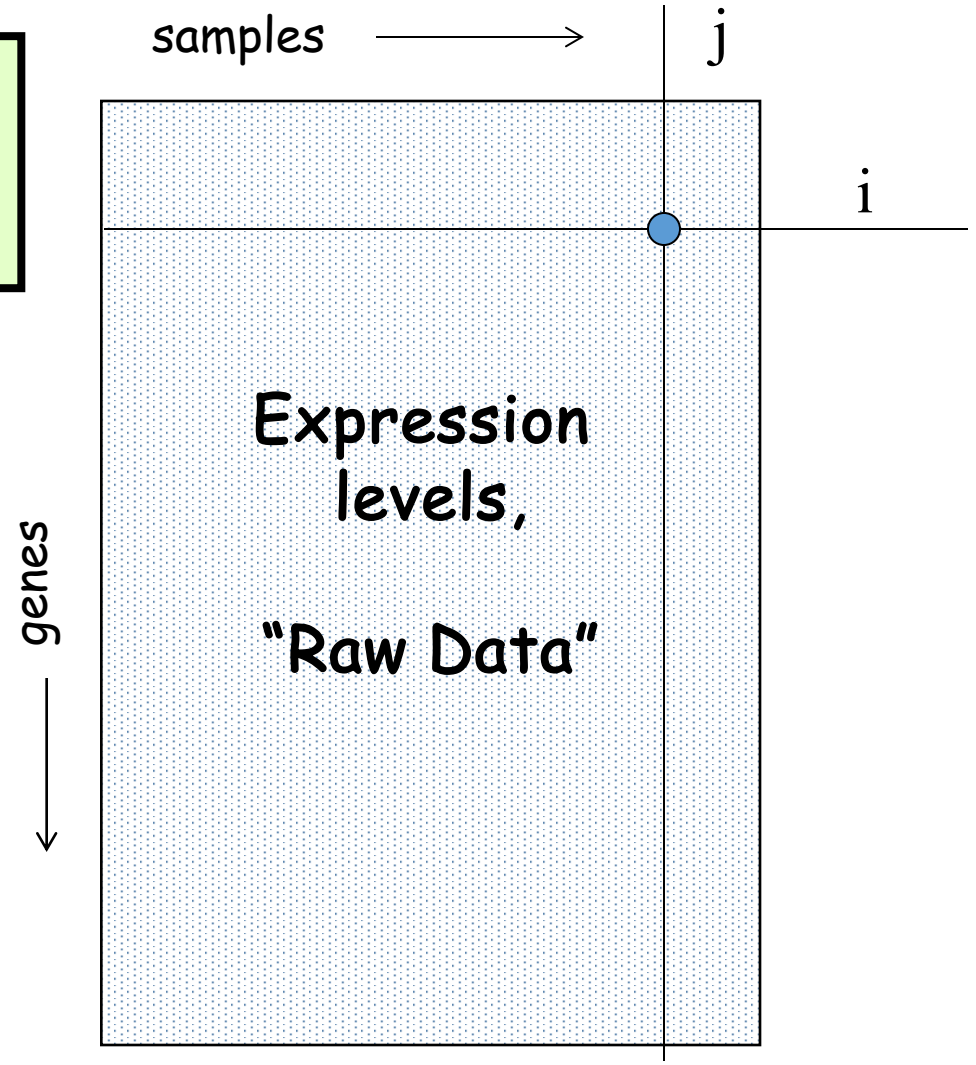
Gene Expression Data, Matrix Representation

$E(i,j)$ represents the expression level of the gene indexed by i
in the experiment/sample indexed by j.



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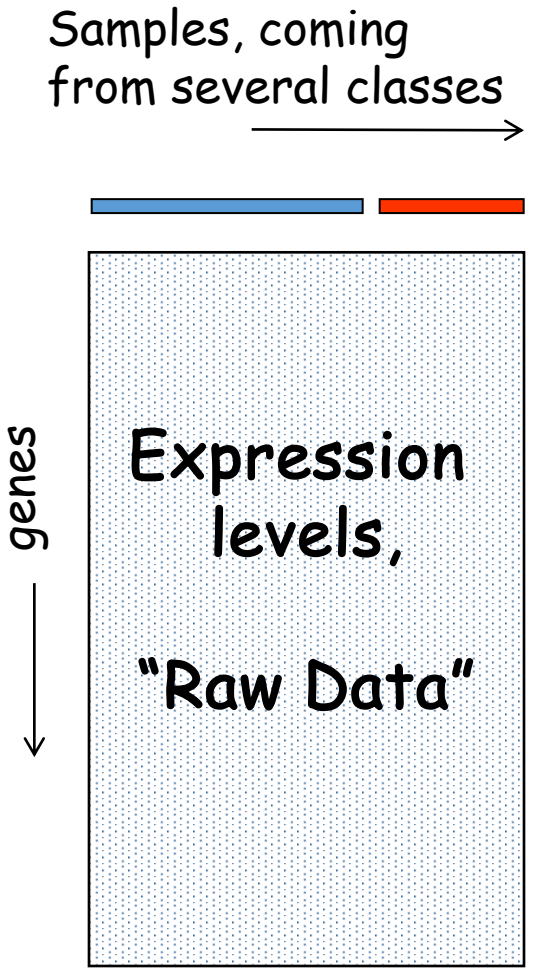
Classified Gene Expression Data, Informative Genes

- Examples:
  - Tumor vs Normal
  - Subtypes of a pathology
  - Prognosis (responders vs non-responders)
  - etc
- Informative genes: genes that sharply separate the two classes. They are *DIFFERENTIALLY EXPRESSED*
- How do we assign DE scores to genes?



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Lung Cancer Informative Genes

Dehan et al, (Kaminski Lab at Sheba), Lung Cancer 2007

- 24 tumors (various types and origins)
- 10 normals (normal edges and normal lung pools)

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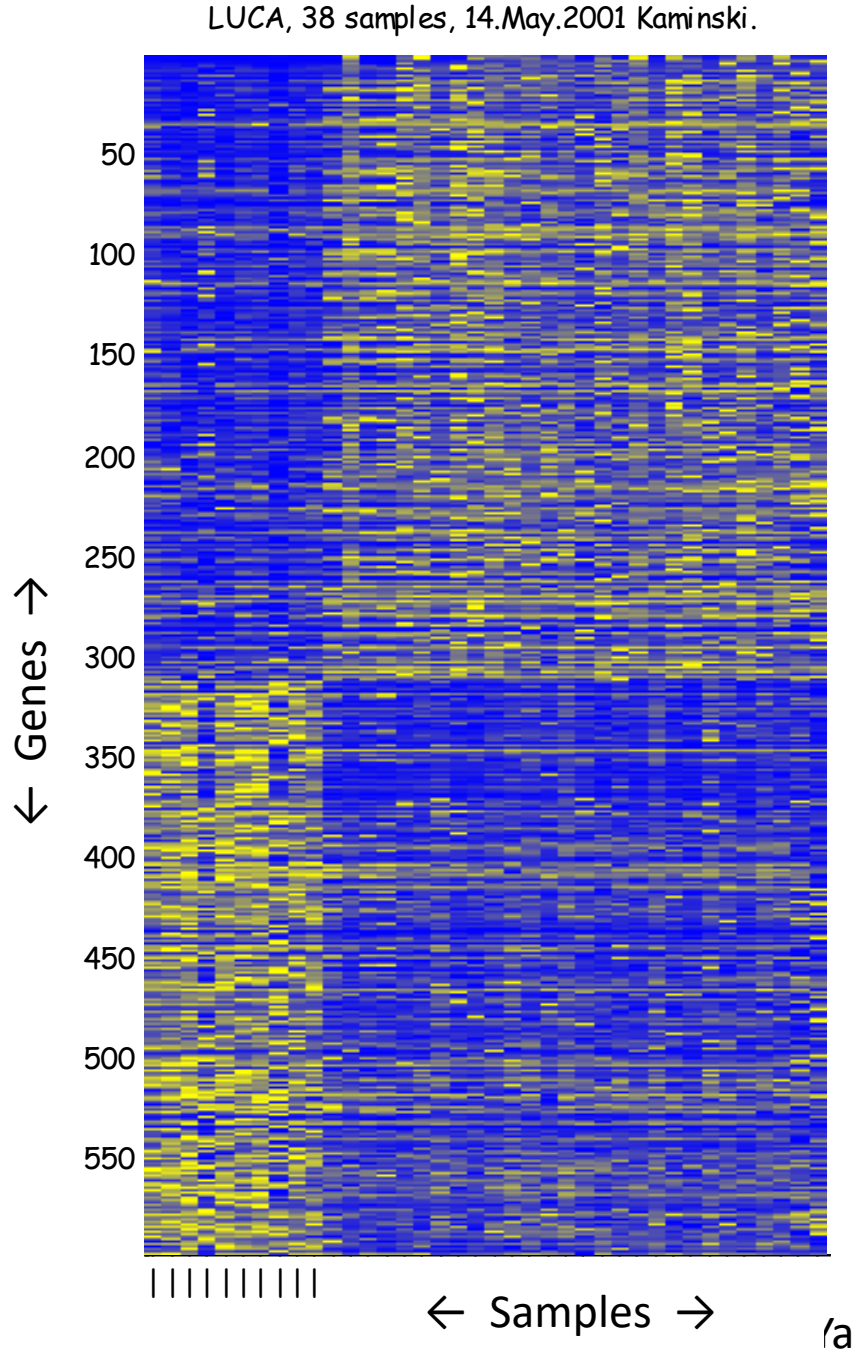

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Wilcoxon Rank Sum test

- Compute the sum of the ranks of the +s:

+	+	–	–	+	+	+	–	–	–	–	–	+	–	–
a1	a2	a3	a4	a5	a6	a7	a8	a9	a10	a11	a12	a13	a14	a15

- In this case:

  RS(+) = 1+2+5+6+7+13 = 34

- The null model: all +/- configurations are equiprobable
- The mean value under the null model is E(RS(+)) = ?
- The p-value of the deviation can be computed using normal approximations

Wilcoxon example

+ + + + + − − − − − − − − − −

RS(+) = ?, p-val = ?

+ + + − − − − − + − − − + − −

RS(+) = ? , p-val = ?

E(RS(+)) = ?

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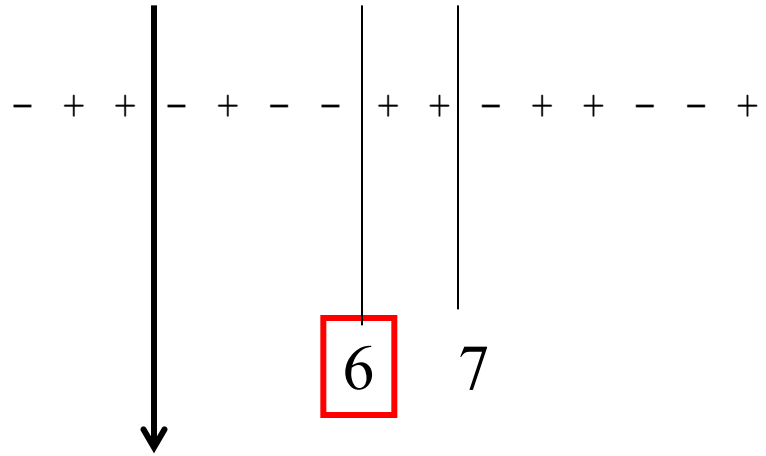
Threshold Error Rate (TNoM) Score

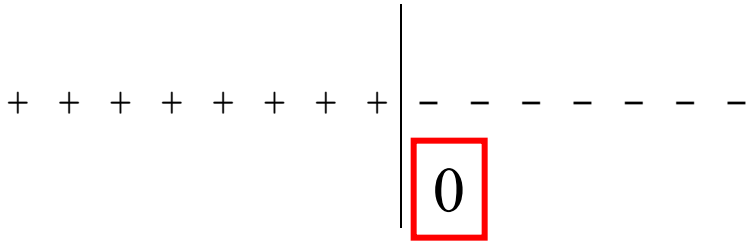
Find the threshold that best separates tumors from normals, count the number of errors committed there.

Ex 1:



# of errors = min(7,8) = 7.

Ex 2: A perfect single gene classifier gets a score of 0.



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Threshold Error Rate (TNoM) Score

Consider the ordered vectors of labels below:

v1 = 0 0 0 0 1 0 0 0 1 1 1 0

v2 = 0 0 1 0 0 1 0 0 0 1 1 0

Compute the TNoM score for these.

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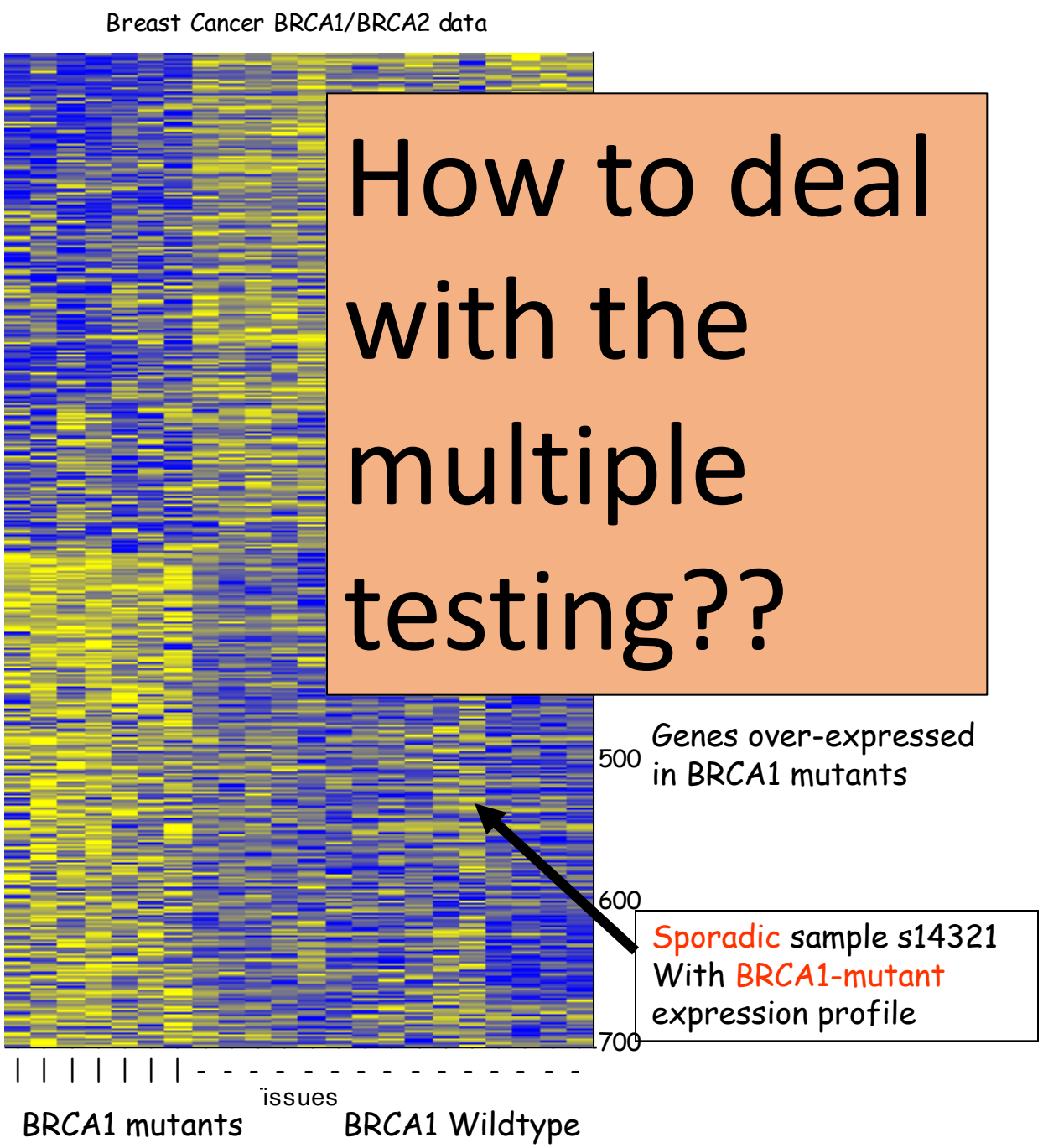
BRCA1 Differential Expression

Collab with NIH; Hedenfalk et al, NEJM 2001



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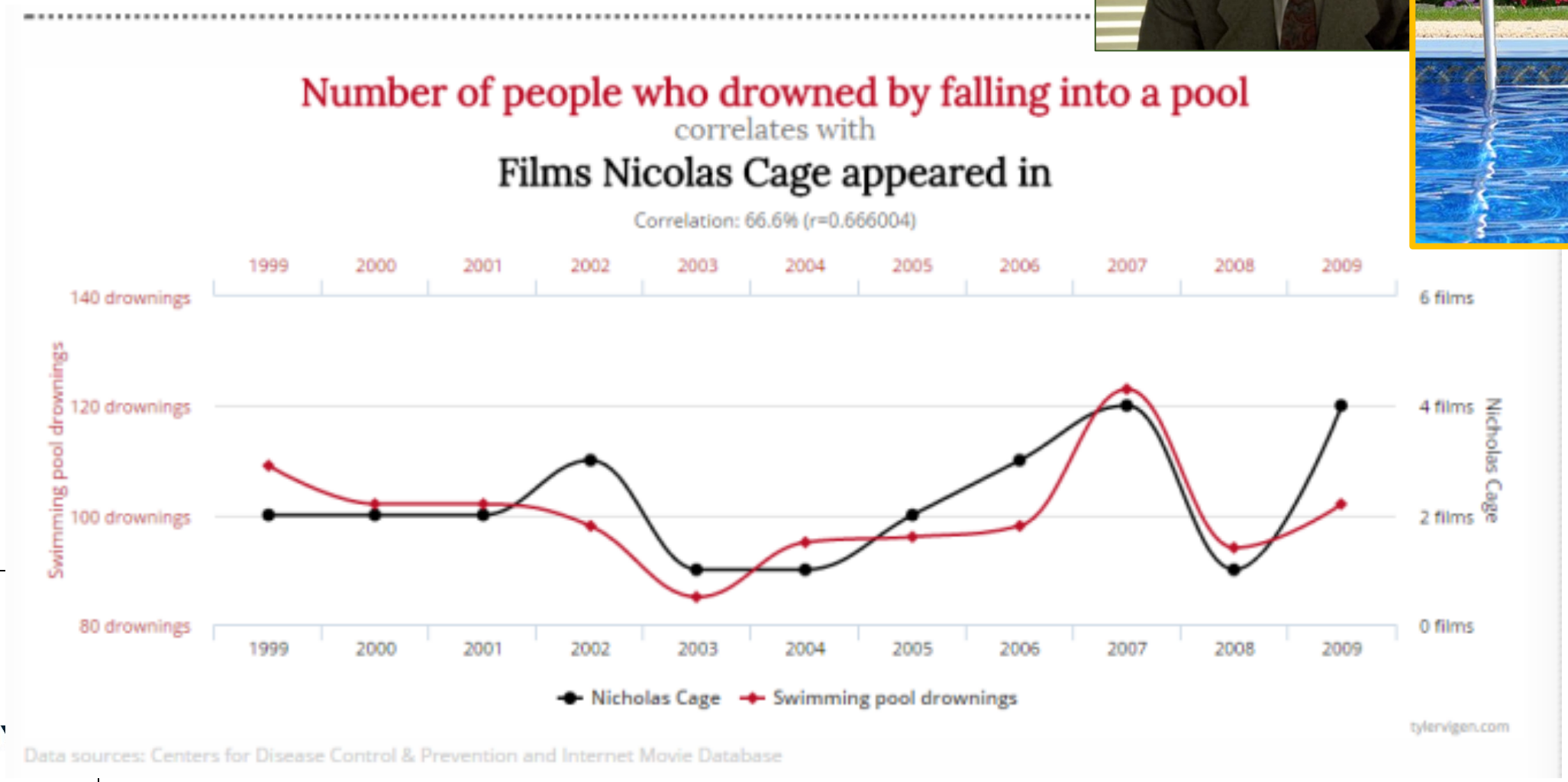
Spurious Correlations



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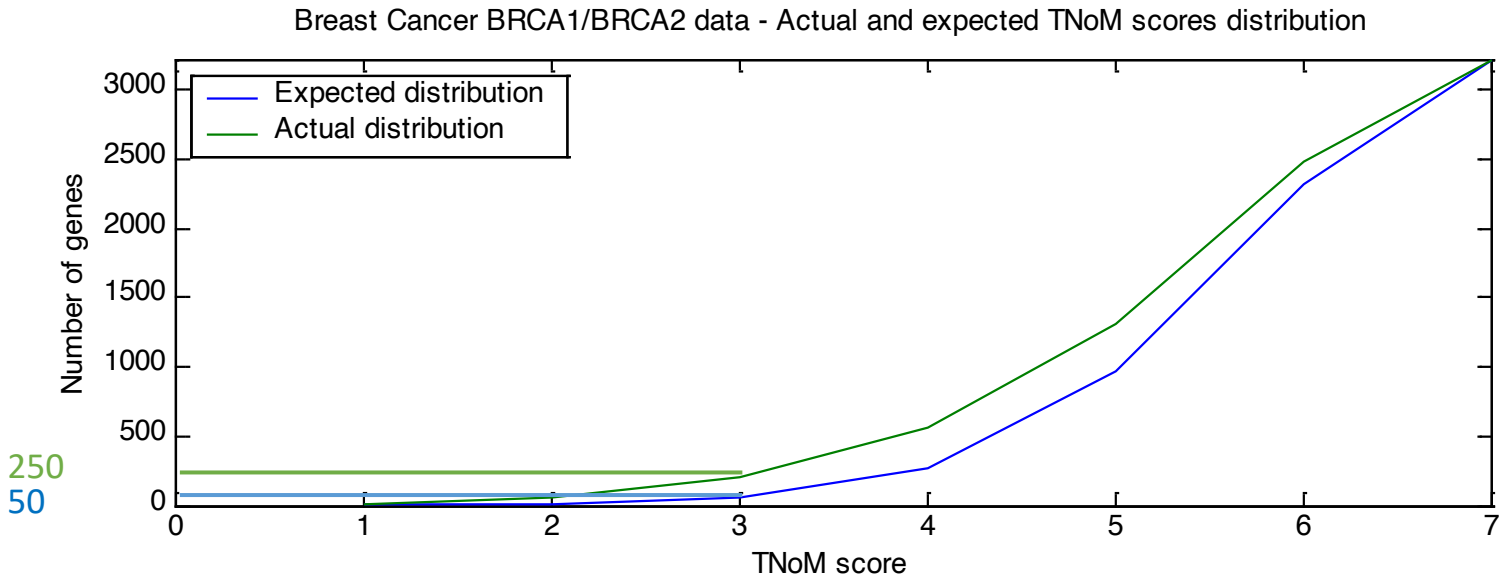
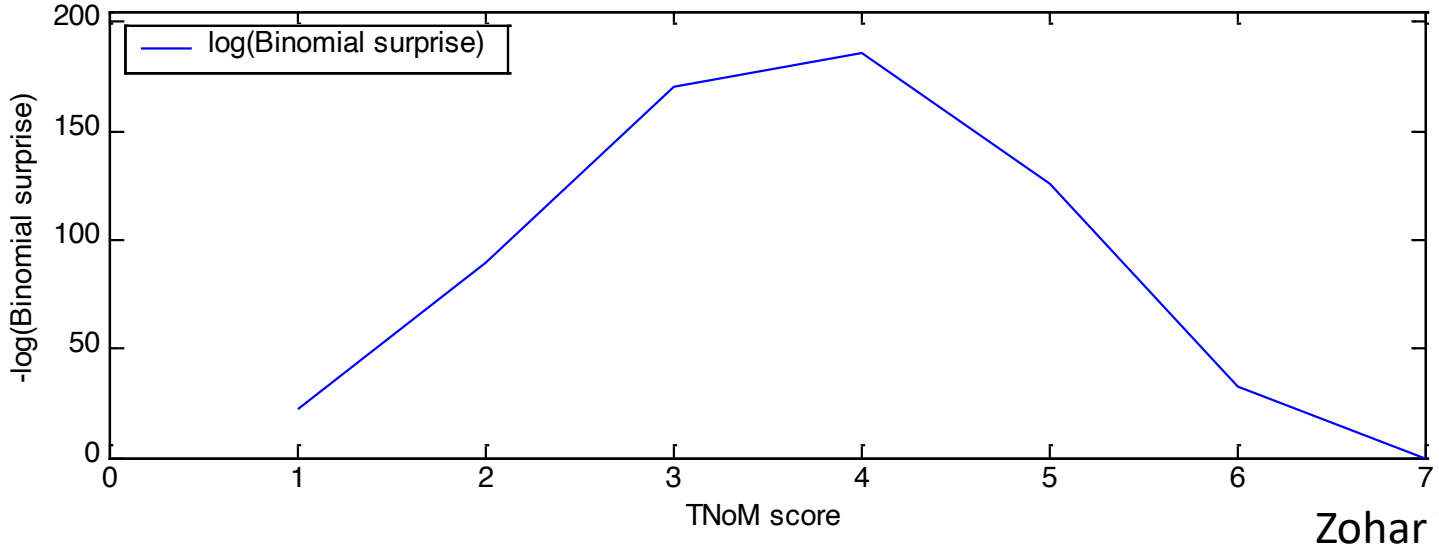
BRCA1 Differential Expression: Overabundance Analysis



$$TNoM(3) \sim Binomial(pval(3), 3000)$$
$$P(TNoM(3) \geq 250) = ?$$



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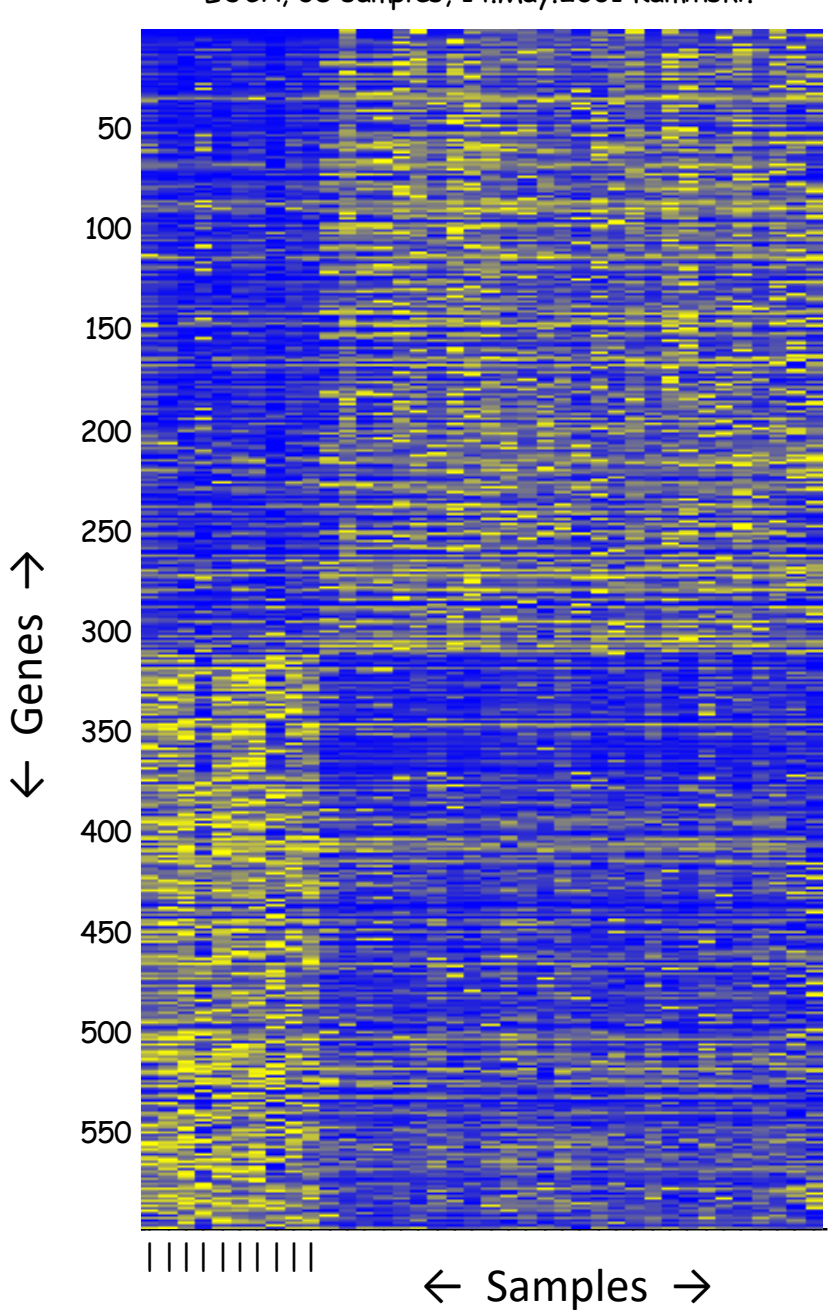

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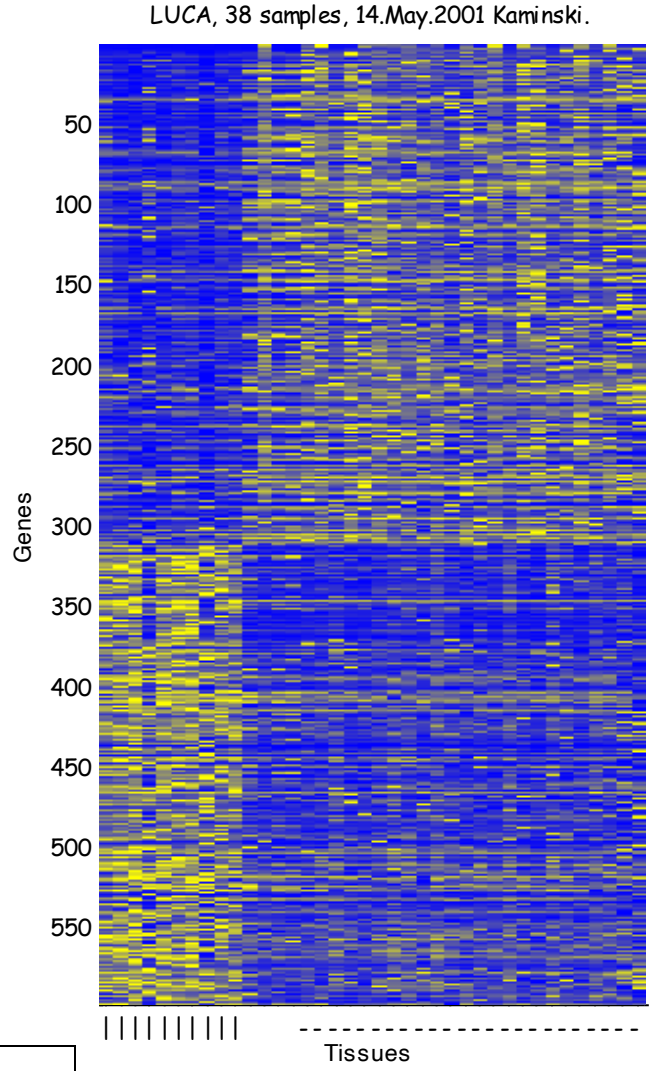
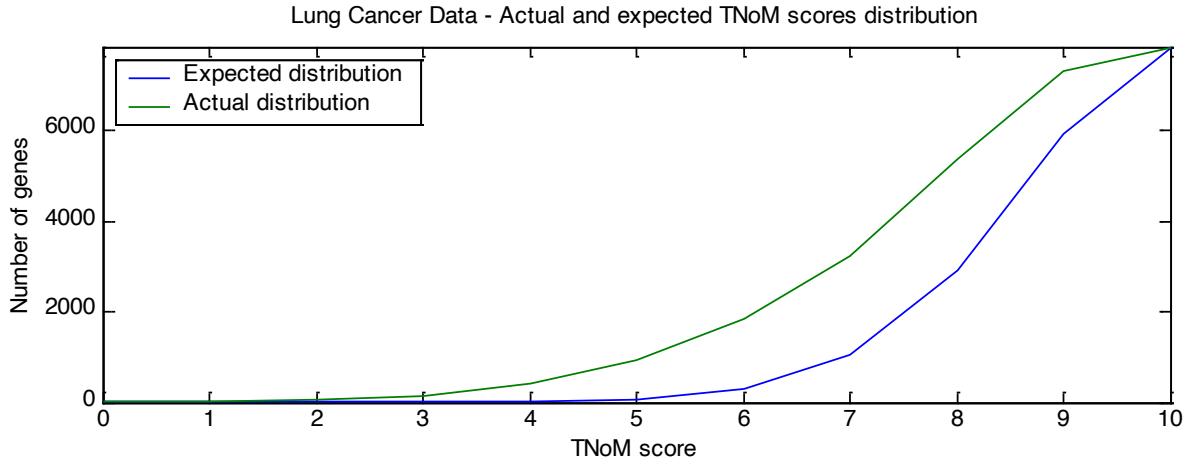
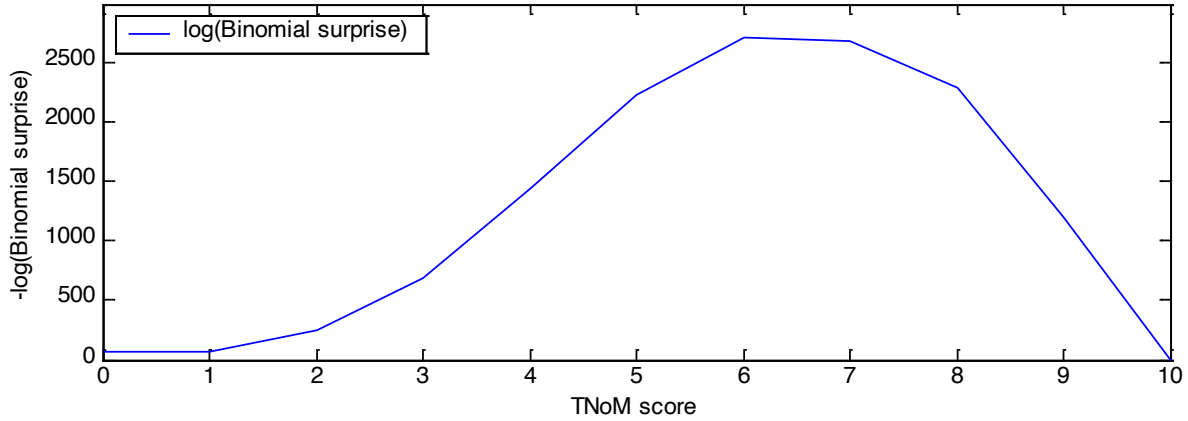


Lung Cancer Overabundance Analysis





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Tall people, Bonferroni

- What is the probability that the person sitting next to you on the bus this evening is >1.90m tall?
- What is the probability of SOME person in the bus being >1.90m tall?
- What is the probability that someone on the IDC campus is >1.90m tall?

- What is a possible naïve (and not very tight) correction? p/N

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Bonferroni - cont

What is the probability of rejecting at least one TRUE hypothesis (each test is one hypothesis testing)? (FWER – Family-Wise Error Rate)

The probability if rejecting a specific TRUE hypothesis is at most p/N.

$$\mathrm{FWER} = P\left\{\bigcup_{i=1}^{N_0}\left(p_i \le \frac{\alpha}{N}\right)\right\} \le \sum_{i=1}^{N_0}\left\{P\left(p_i \le \frac{\alpha}{N}\right)\right\} = N_0\frac{\alpha}{N} \le \alpha$$

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Bonferroni - cont

Assuming that we profiled 20K genes.

Is a DE p-value of $5 \cdot 10^{-6}$ significant (at 0.05)?

How many genes with a p-value at most $5 \cdot 10^{-6}$ are we expecting to see?

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How are p-values distributed under the null model???

- Single Experiment:

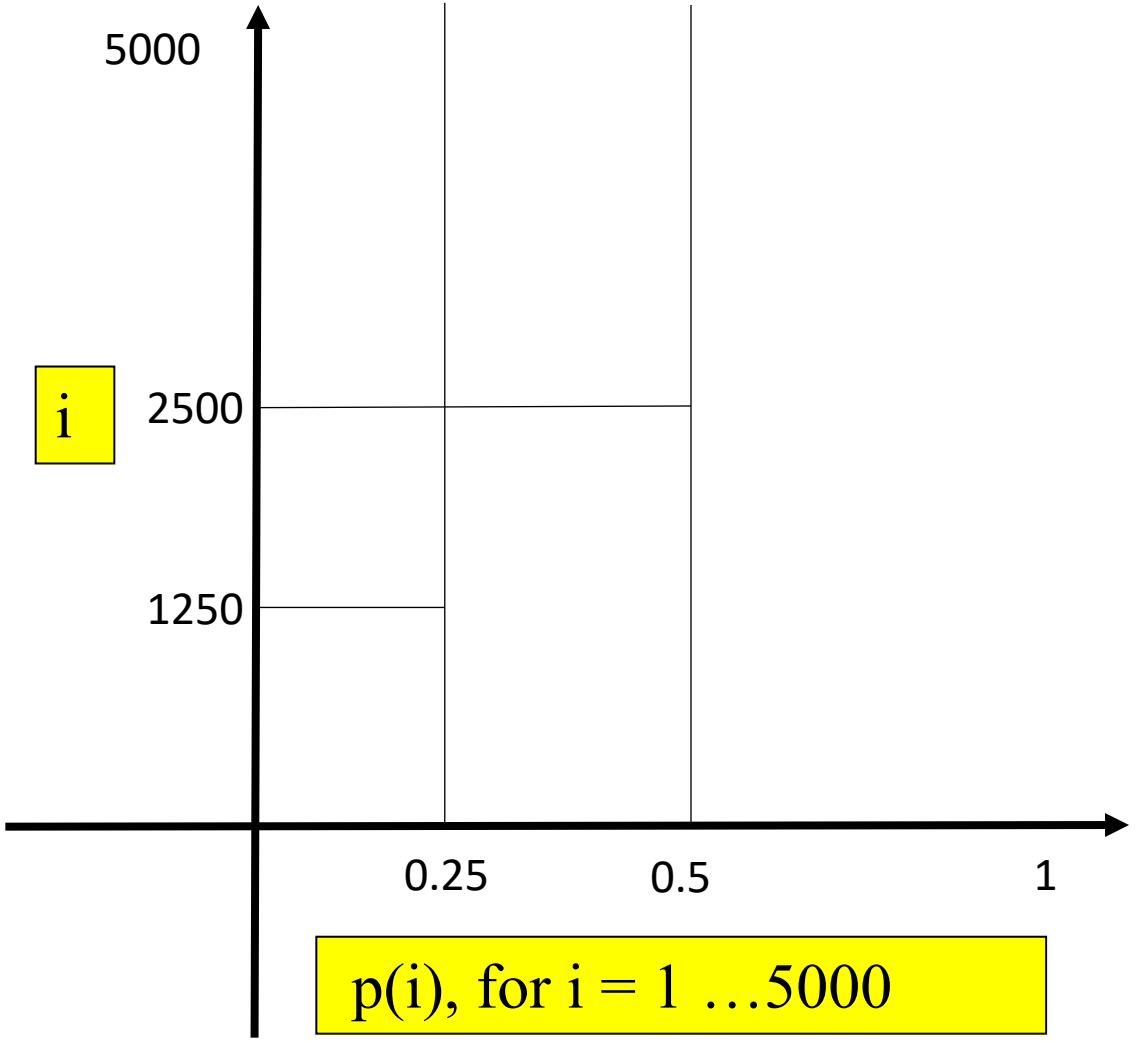
1. Toss a fair coin 100 times
2. Compute $\hat{\theta} = \frac{\#1}{100}$
3. Compute the (one sided **left**) p-value of $\hat{\theta}$ under the fair coin null model $\mathrm{P}(X \leq \#1)$

$$X \sim Binom(0.5,100)$$

- Repeat 5000 times:

$$\left(\hat{\theta}(1), p(1)\right), \left(\hat{\theta}(2), p(2)\right), \ldots, \left(\hat{\theta}(5000), p(5000)\right)$$

$$p(1) \leq p(2) \leq \cdots p(5000)$$



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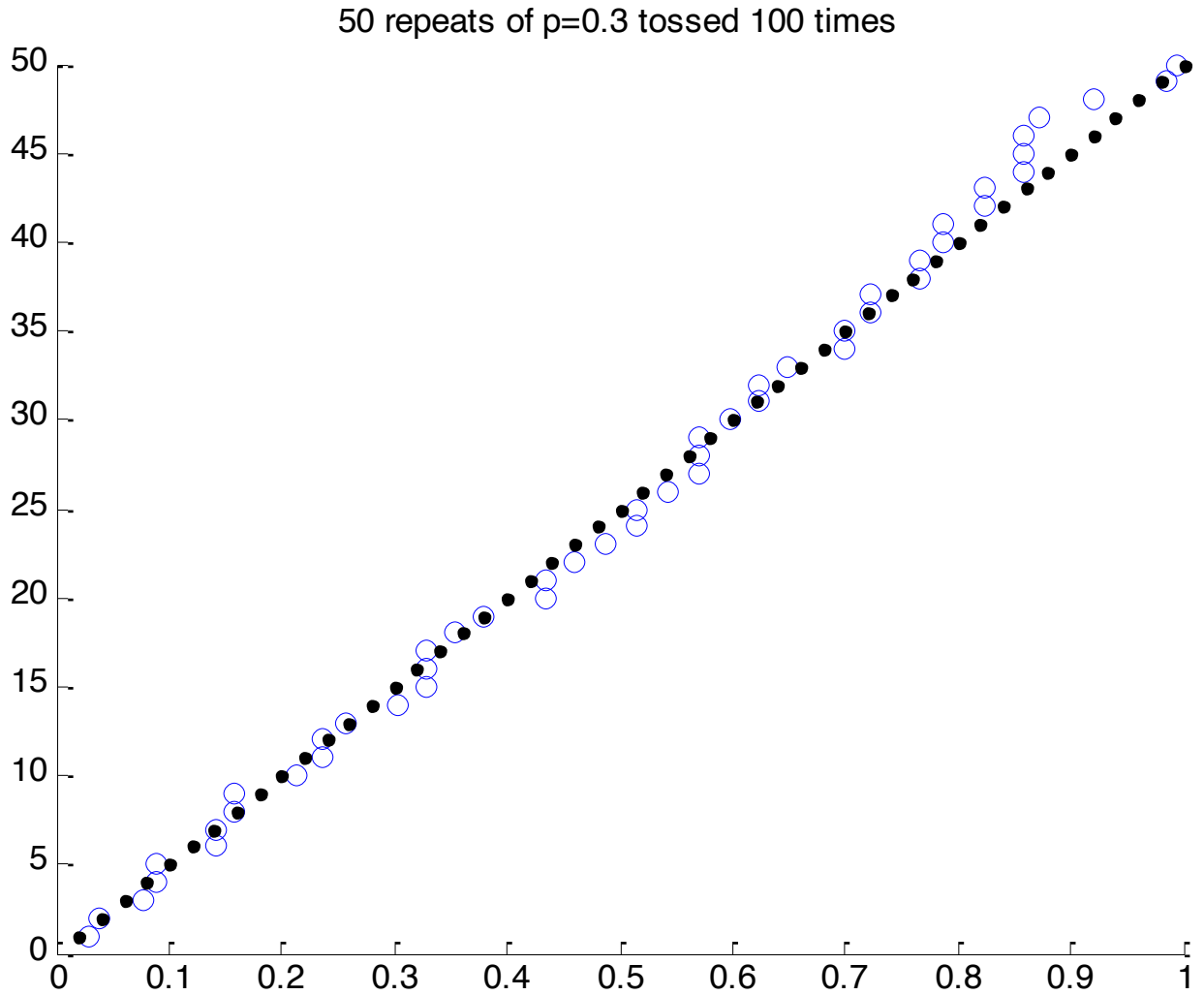
Distribution of p-values under the null model

- Generating data using a coin with $\boldsymbol{p = 0.3}$
- under a null model of $\boldsymbol{p = 0.3}$



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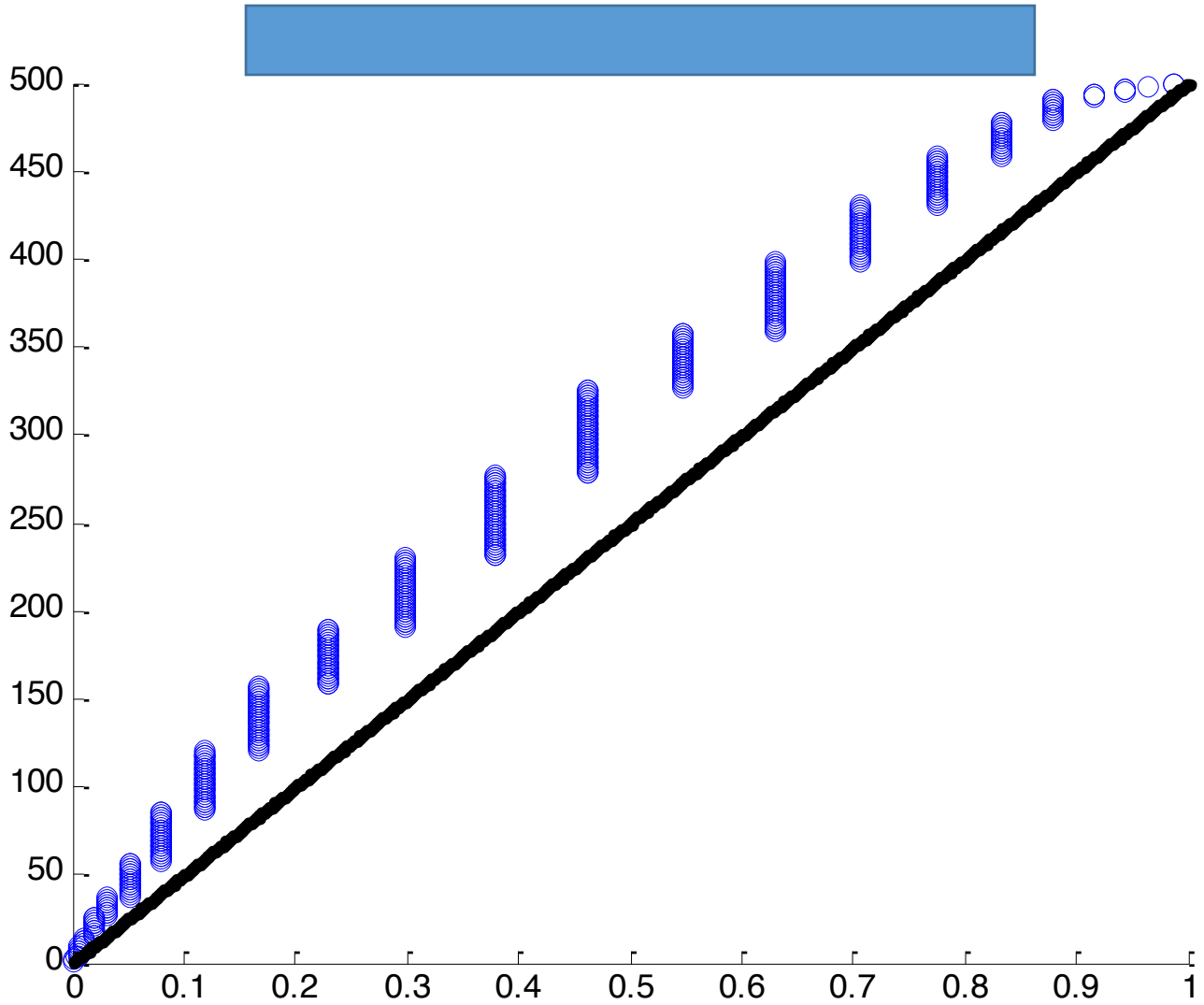
Distribution of p-values under the null model

$$\mathrm{P}(X \leq \#1)$$

- Generating data using a coin with $\boldsymbol{p = 0.3}$
- under a null model of $\boldsymbol{p = 0.32}$



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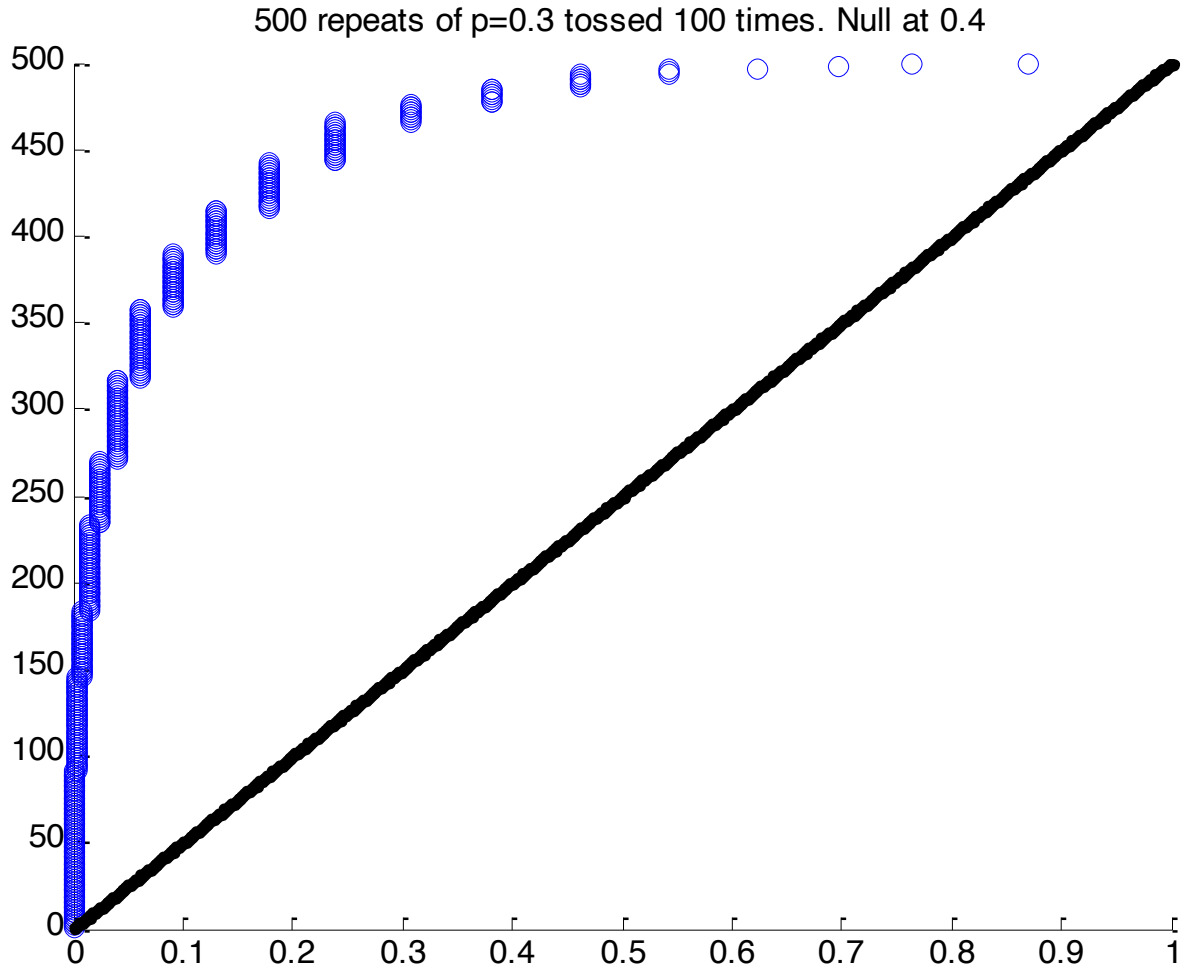
Distribution of p-values under the null model

$$\mathrm{P}(X \leq \#1)$$

- Generating data using a coin with $\boldsymbol{p = 0.3}$
- under a null model of $\boldsymbol{p = 0.4}$



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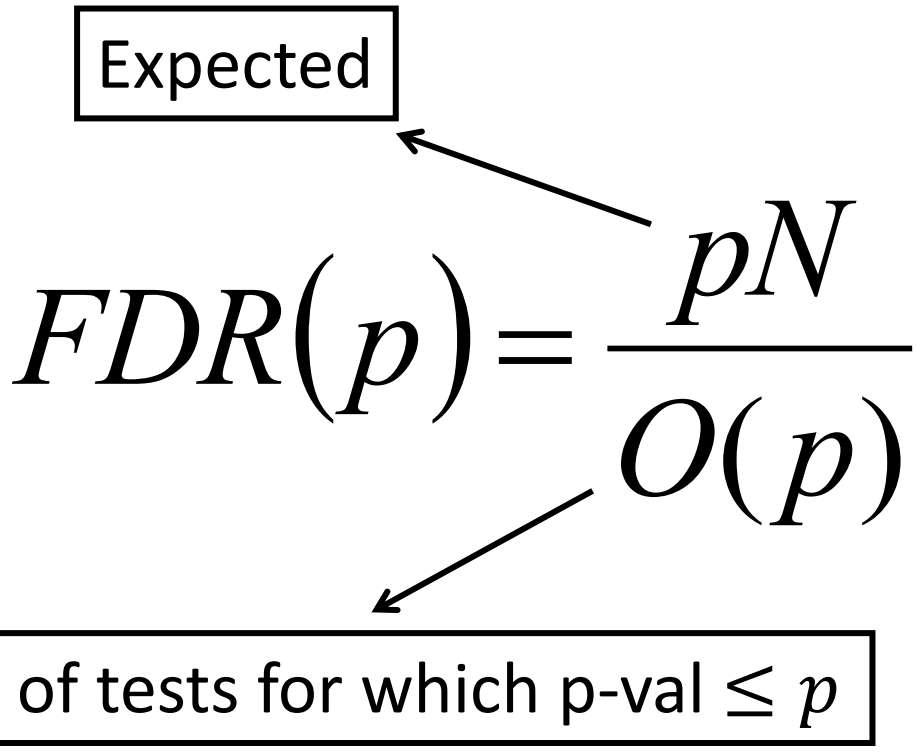
False Discovery Rate (FDR)

What fraction of the observed DE is expected at random (under a null-model)?



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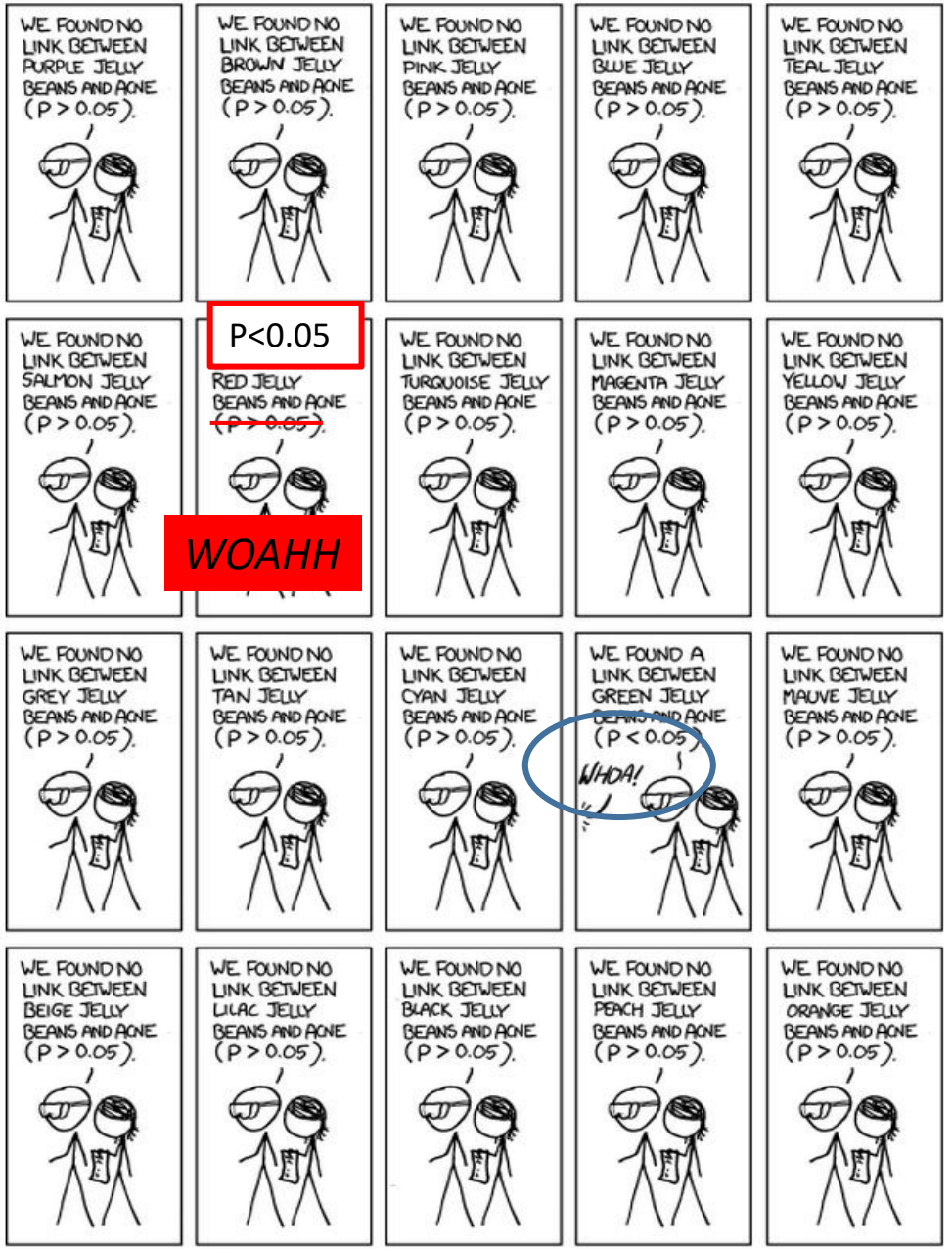
More events

What if two colors were linked at 0.05? Three?



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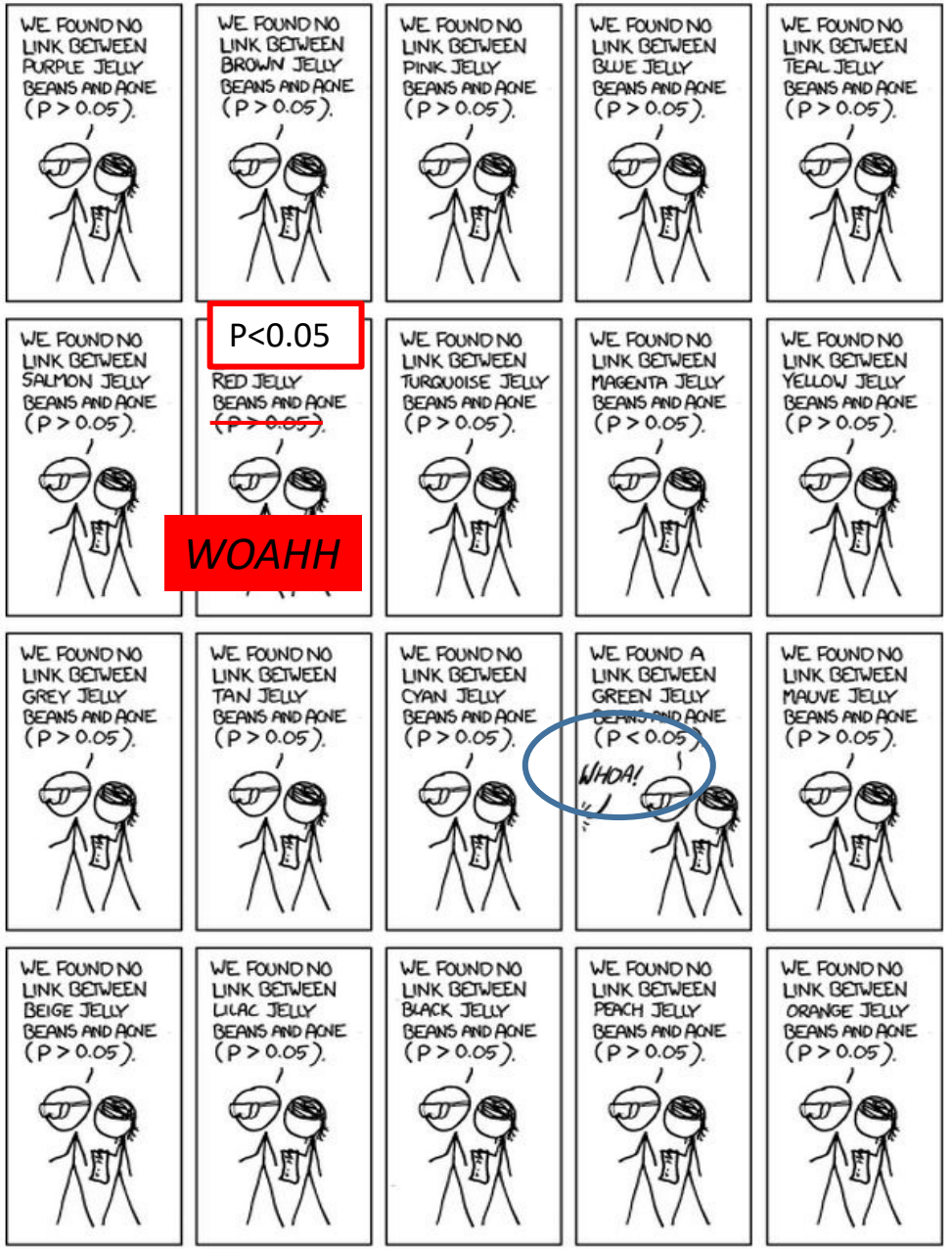
More events

$$FDR(0.05) = ?$$



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FDR at any i

$$FDR(p) = \frac{pN}{O(p)}$$

$$FDR(i) = \frac{p(i)N}{i}$$

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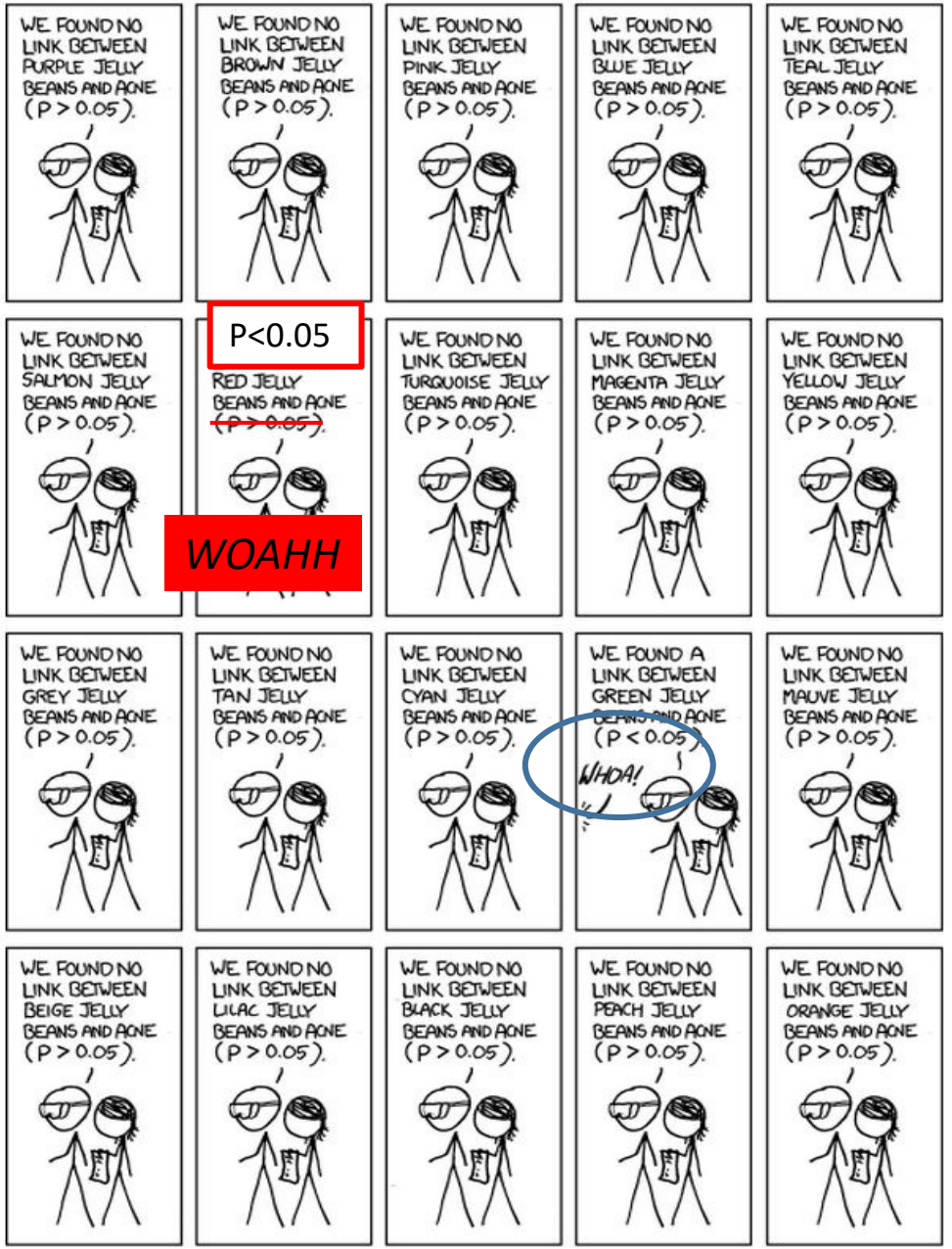
More events

$$FDR(2) = ?$$



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Distribution of p-values under the null model

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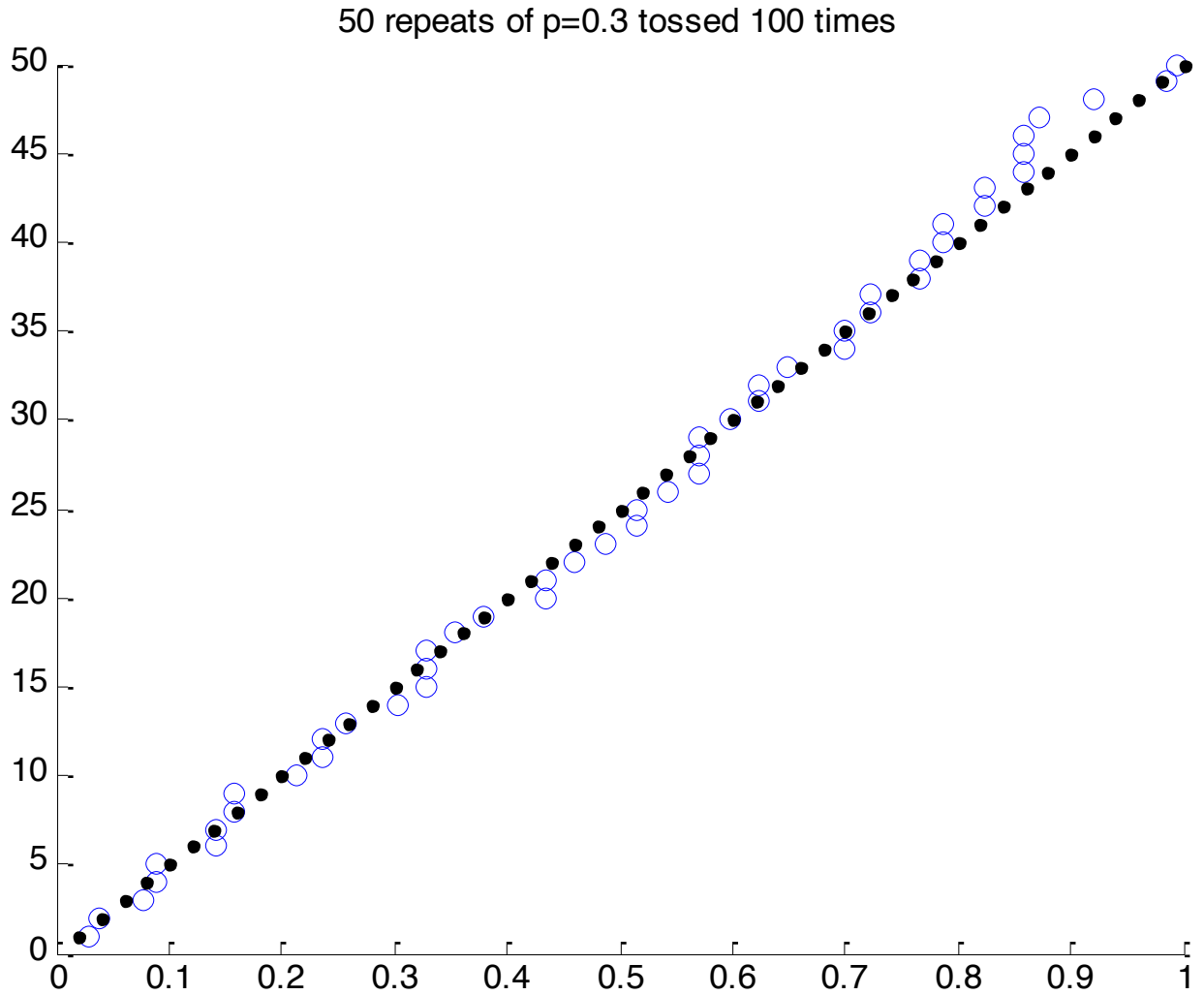
- under a null model of $\boldsymbol{p = 0.3}$



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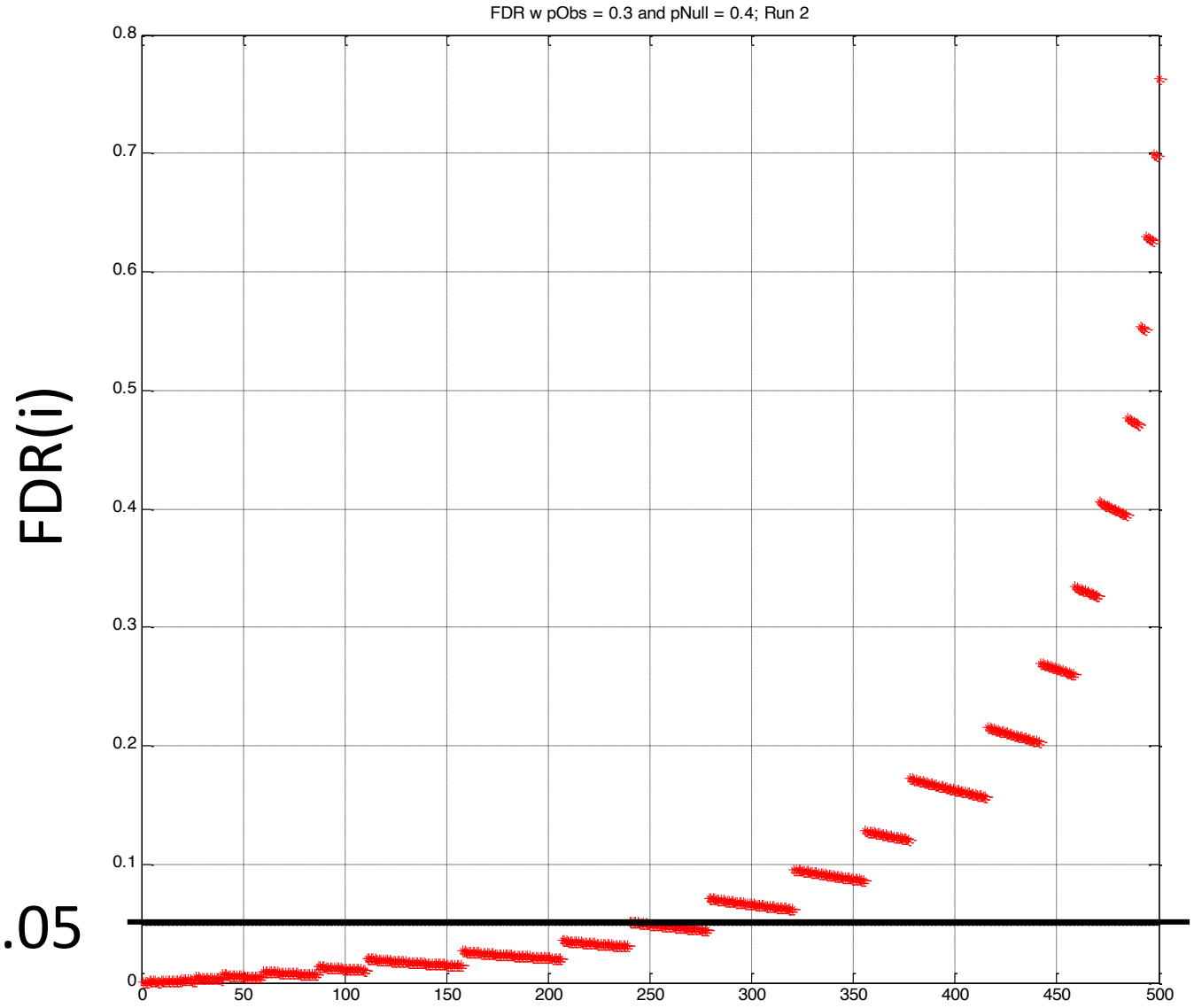
FDR

- Generating data using a coin with $\boldsymbol{p = 0.3}$
- under a null model of $\boldsymbol{p = 0.4}$



$$FDR(i) = p(i) \cdot N / i$$

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A more subtle correction

$$\mathrm{RobFDR}(i) = \min_{j \geq i} \left(\frac{p(j) \cdot N}{j} \right)$$

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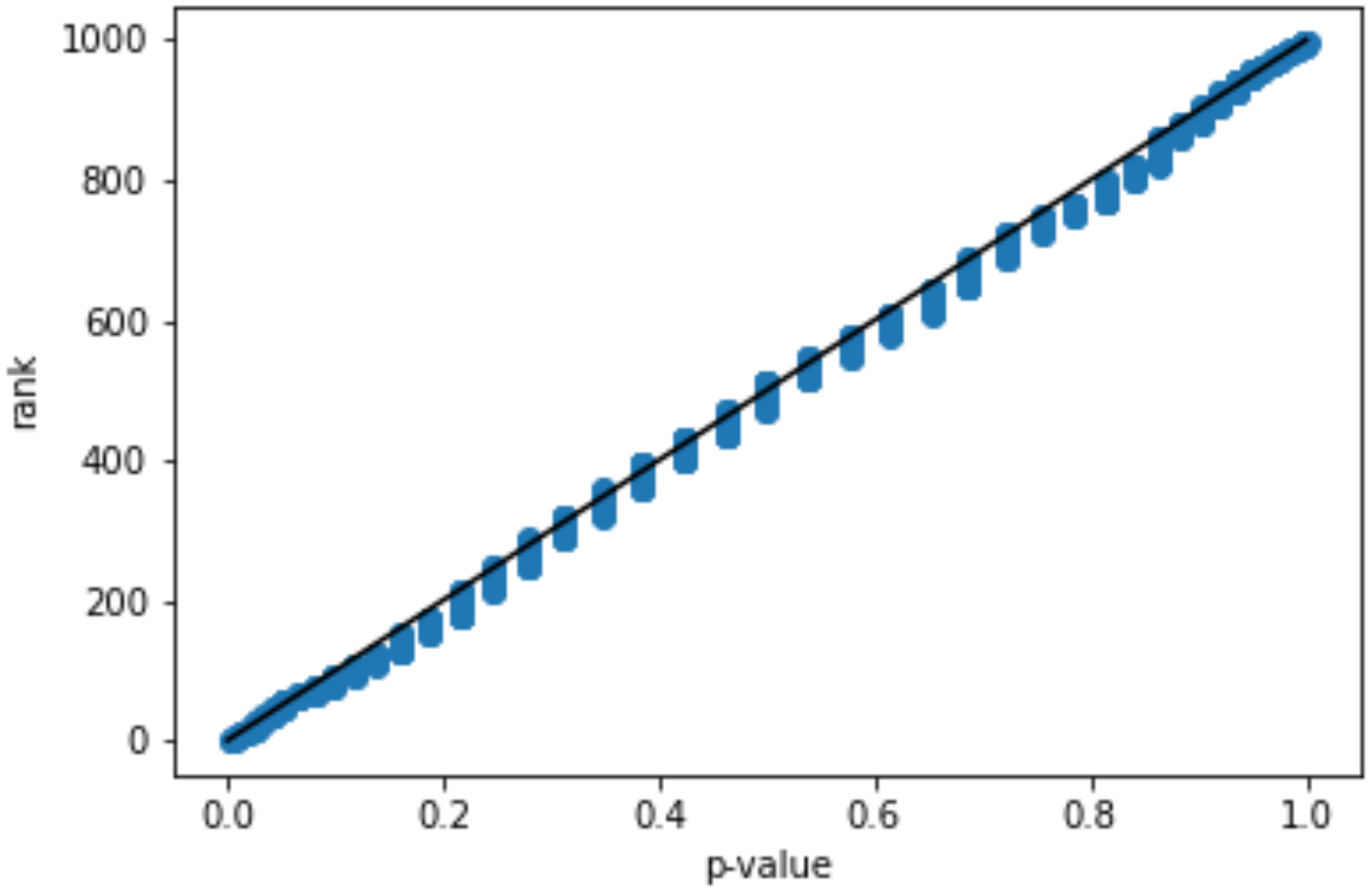
WRS FDR: A ~N(0,1) and B ~ N(0,1), n= 20,20



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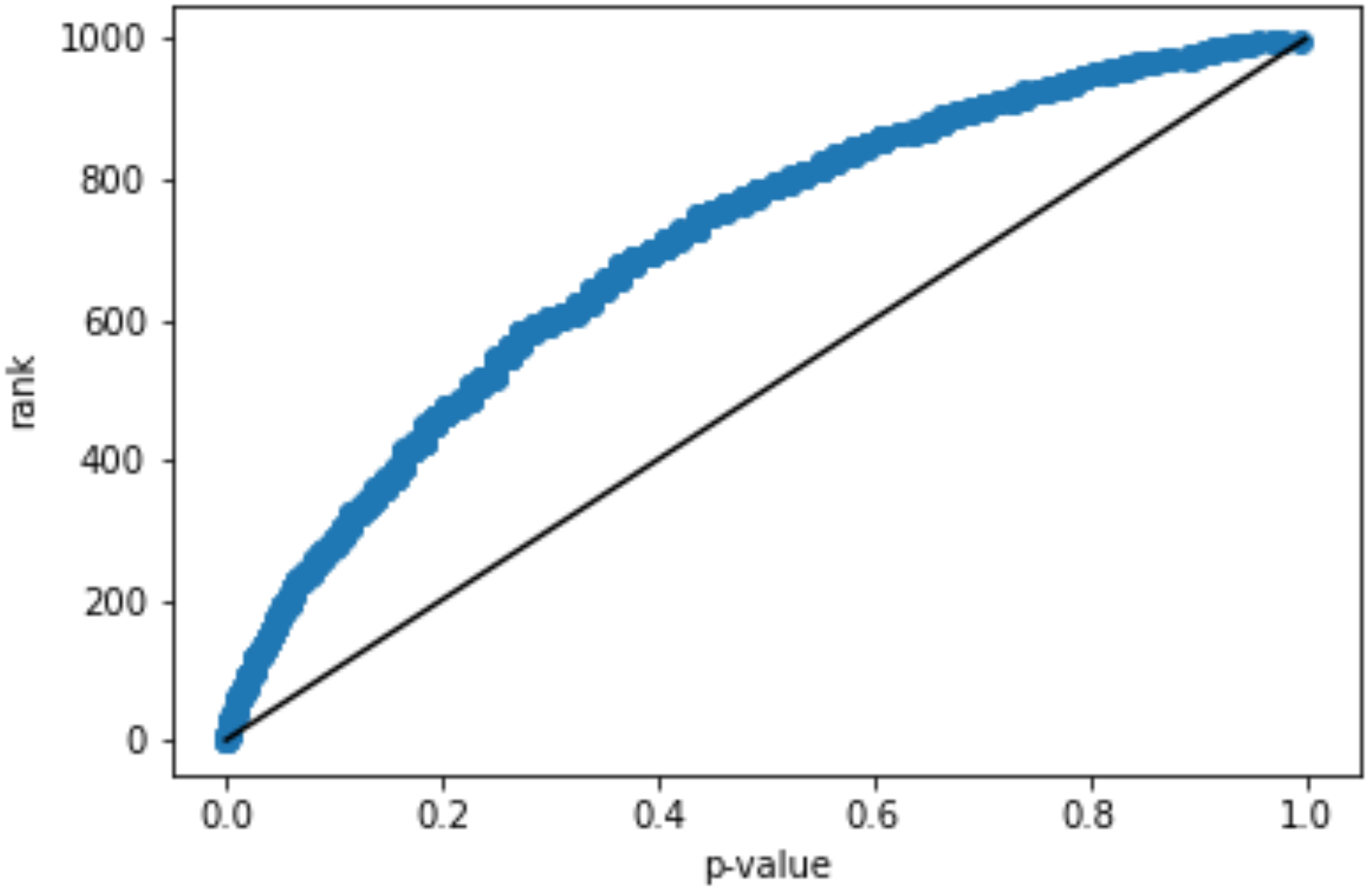
WRS FDR: A ~N(0,1) and B ~ N(0.25,1), n= 20,20



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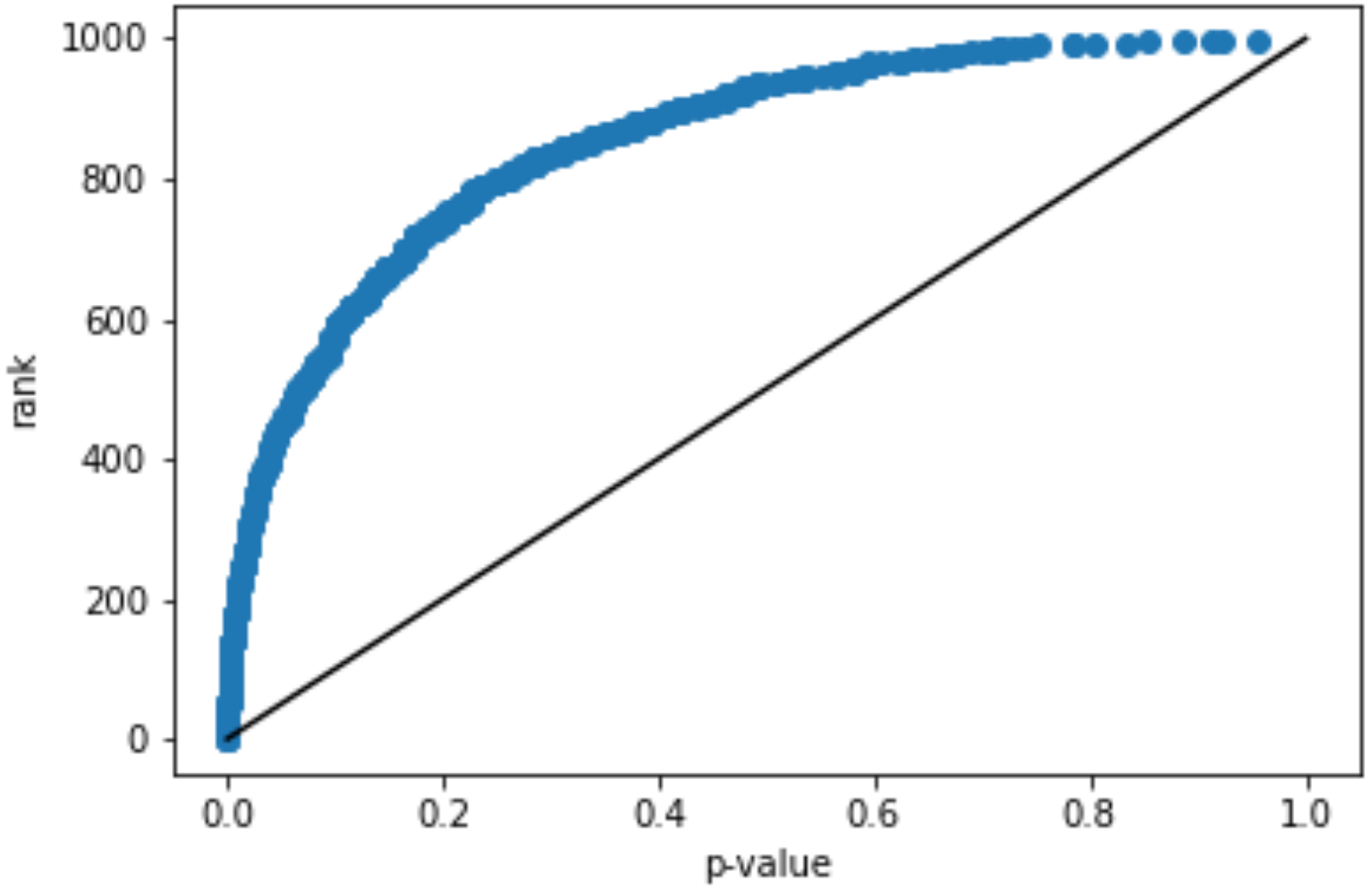
WRS FDR: A ~N(0,1) and B ~ N(?,1), n= 20,20



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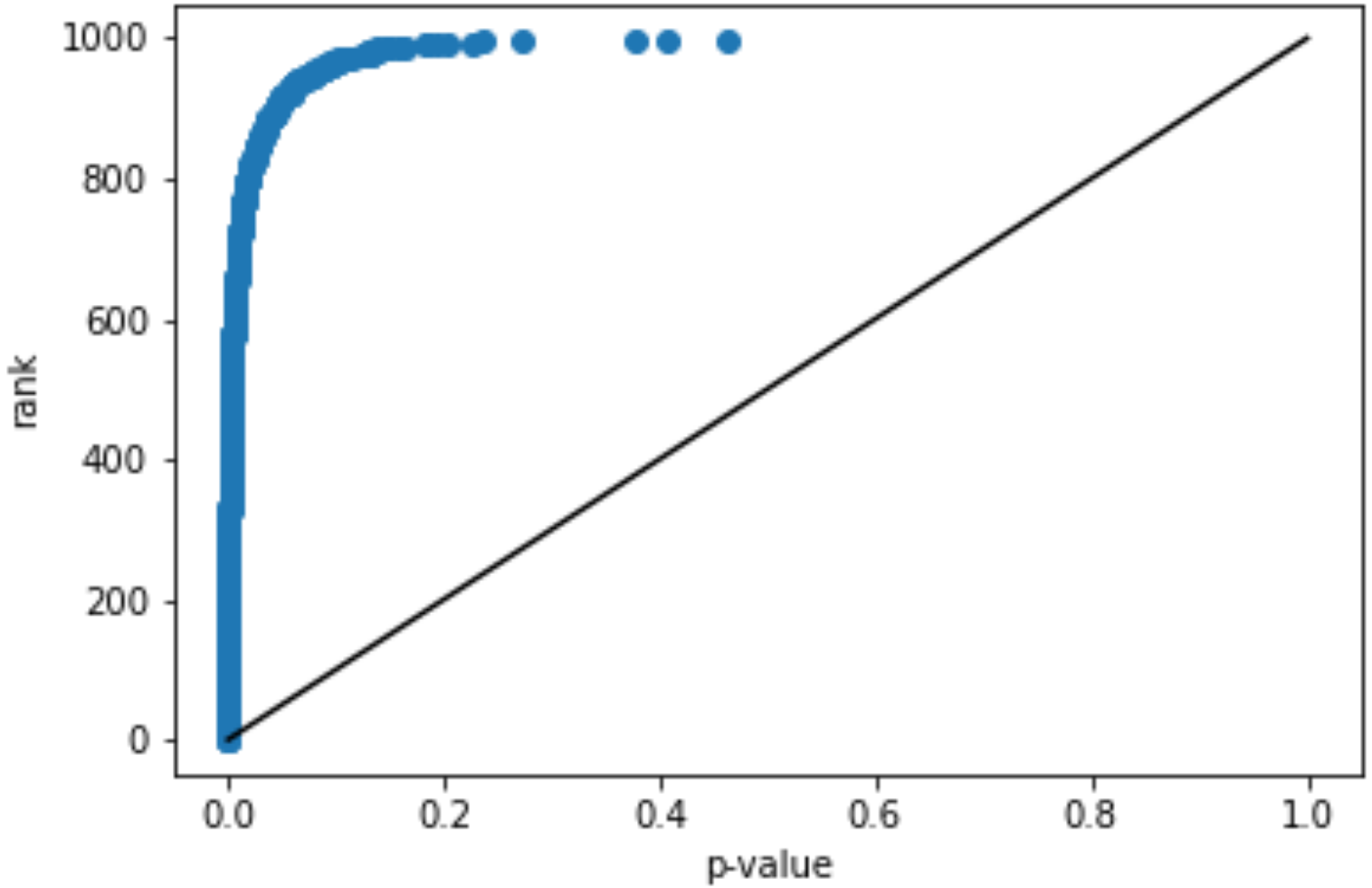
WRS FDR: A ~N(0,1) and B ~ N(1,1), n= 20,20



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FDR – the procedure

Yoav Benjamini and Yosef Hochberg 1995

Replace by the robust version

- Assume that we performed N measurements (comparisons, observations etc)
- Rank the computed significance of the findings:
  $p(1) \le p(2) \le p(3) \le p(4) \le \ldots. \le p(N-1) \le p(N)$
- Under the null model, the expected number of observations with p-value better than p(i) is $p(i) \cdot N$
- The false discovery rate at i is therefore:

$$\mathrm{RobFDR}(i) = \min_{j \ge i} \left(\frac{p(j) \cdot N}{j} \right)$$

- A corrected hypothesis testing in this case would be to find the max i that satisfies $\mathrm{FDR}(i) \le \tau$, where τ (e.g 0.05) is the required false discovery rate.

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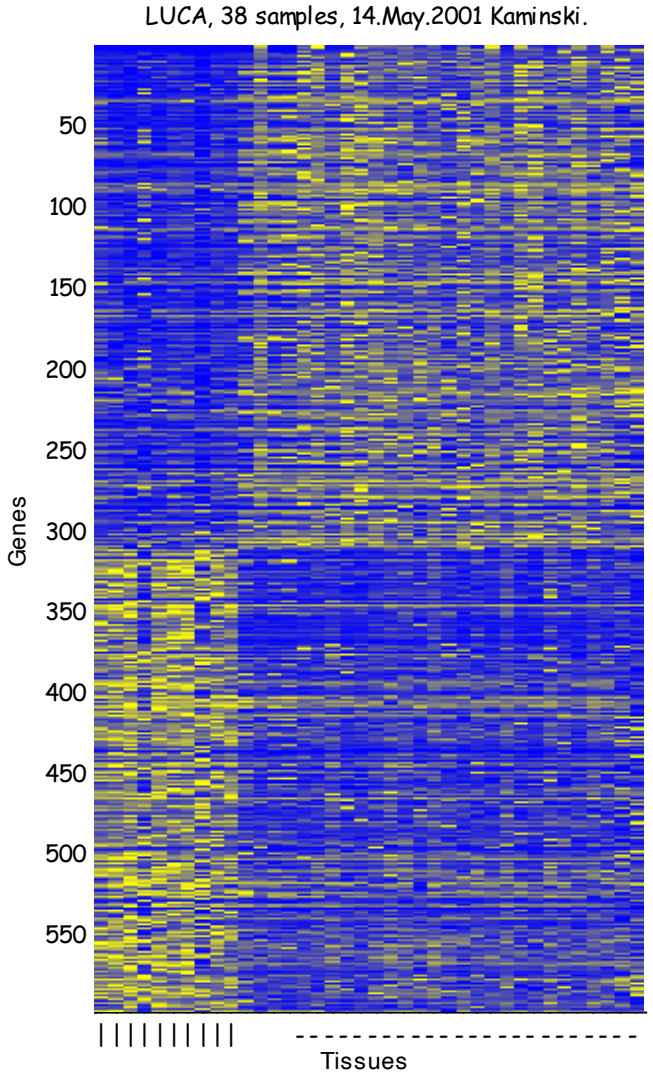
In the context of DGE …



We observed (say) 200 genes at FDR=0.05, using a WRS test

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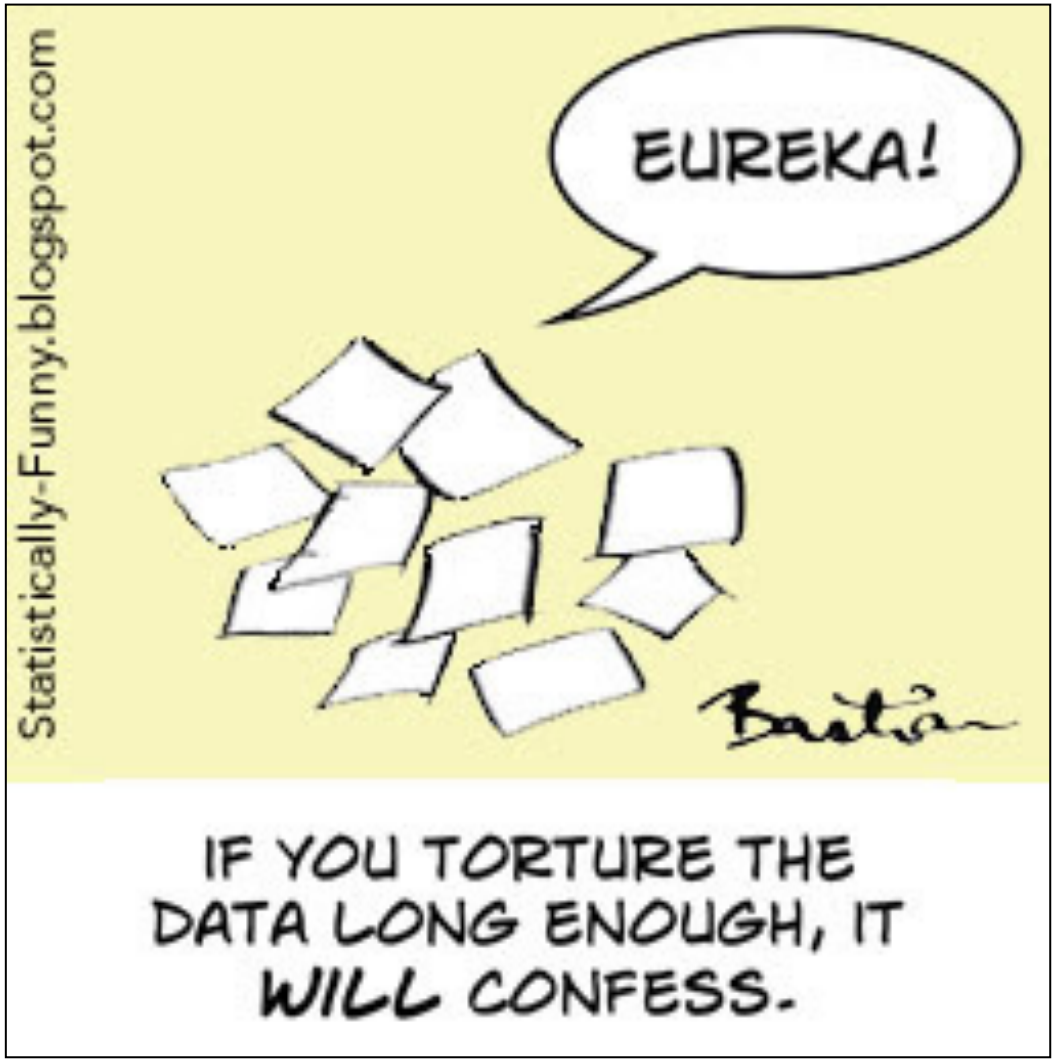
Multiple testing and FDR

- Greater data volumes require more careful inferential statistics approaches.
- Approaches to addressing multiple testing:
  - Bonferroni correction
  - Report FDR results
  - Simulations under a null



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