An Overview of Randomization

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Proteomics Team

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Outline

1. **What is randomization**
2. Why is randomization needed?
3. Types of randomization
4. Checking randomization
5. What can happen when randomization is not well-performed?
6. Conclusions

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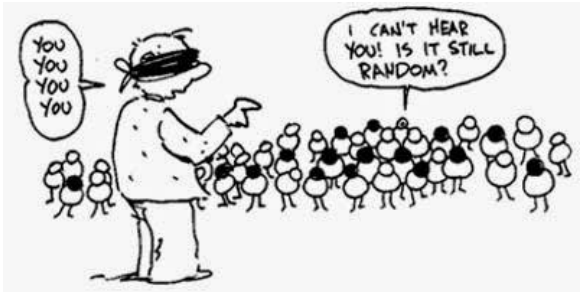
What is randomization – random allocation?

- The process of making something **random** (au hasard, aléatoire).

- In statistics, a random selection is a selection in which every item in a set has the same probability of being chosen.

- Typically, **samples** (échantillons) assigned to **batches** (lots, groupes d'expériences / d'échantillons / de sujets) or **subjects** assigned to treatments / arms.

- **Blinding**
  (à l'aveugle)



http://testofhypothesis.blogspot.com/2014/09/the-sample.html

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Why is randomization needed?

To minimize / control / reduce bias

- Unbalanced samples
- Sample is not representative of the general "population"
- Measurements are not consistent over time or spatially
  - Machine calibration drifts over time
  - Person learns or gets tired over time
  - Multiple persons perform the measurements
- Patients / samples are not consistent over time
  - Patients follow less strictly the protocol
  - Samples degrade the longer they are stored
  - New people are enrolled over time

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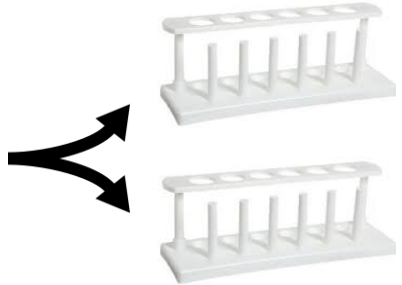
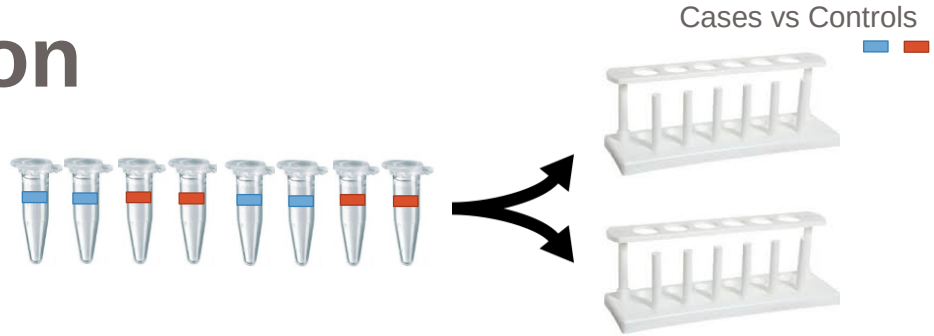
Types of randomization

- **Simple randomization**



- Block randomization
- Stratified randomization
- Adaptive randomization

Types of randomization



- Simple randomization
- **Block randomization**

Size of block: 4, 2 cases and 2 controls in each block

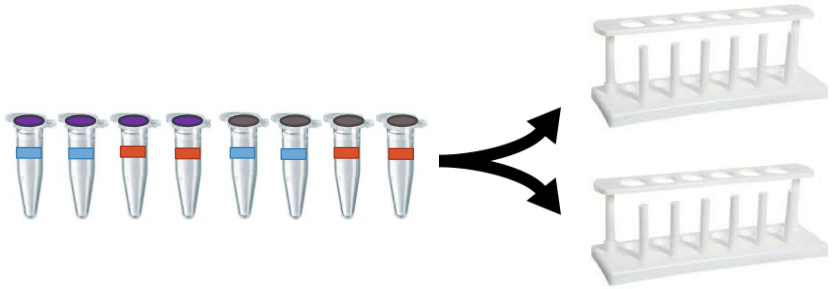
- Stratified randomization
- Adaptive randomization

Types of randomization

2 genders ♀ ♂

Cases vs Controls

- Simple randomization
- Block randomization



- **Stratified randomization**

Size of block: 2, 1 case and 1 control in each block, for each gender

♀ ♂ …

- Adaptive randomization

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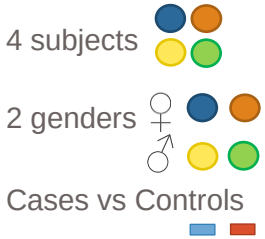
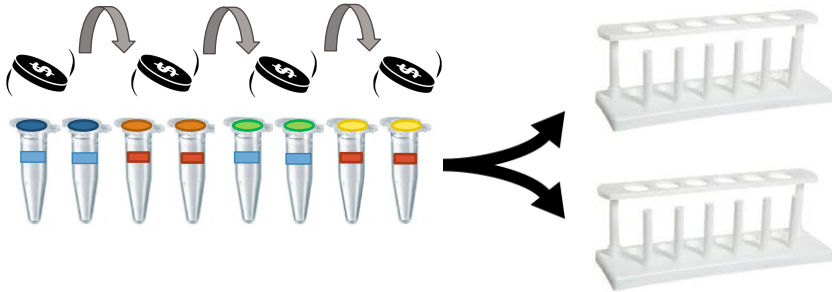
Types of randomization



- Simple randomization
- Block randomization
- Stratified randomization
- **Adaptive randomization**



First randomize, then perform assignment, then adapt the randomization according to results of previously analyzed samples or to additional variables of the samples in each batch

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Checking randomization

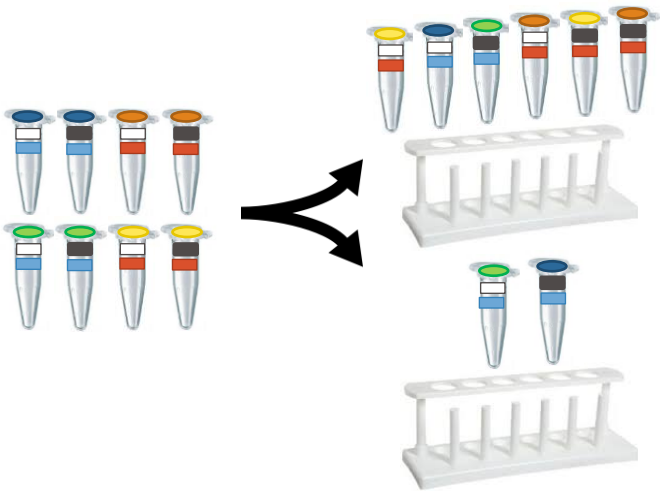
4 subjects

2 genders

2 time points

Cases vs Controls

A



B

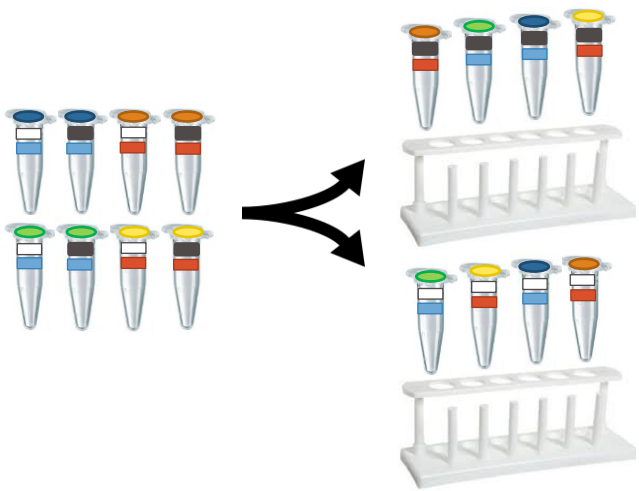


Plate	Females / Males	Time Points 1 / 2	Cases / Controls	N
1	3/3	3/3	2/4	6
2	1/1	1/1	2/0	2

Plate	Females / Males	Time Points 1 / 2	Cases / Controls	N
1	2/2	0/4	2/2	4
2	2/2	4/0	2/2	4

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Checking randomization

4 subjects

2 genders

2 time points

Cases vs Controls

C

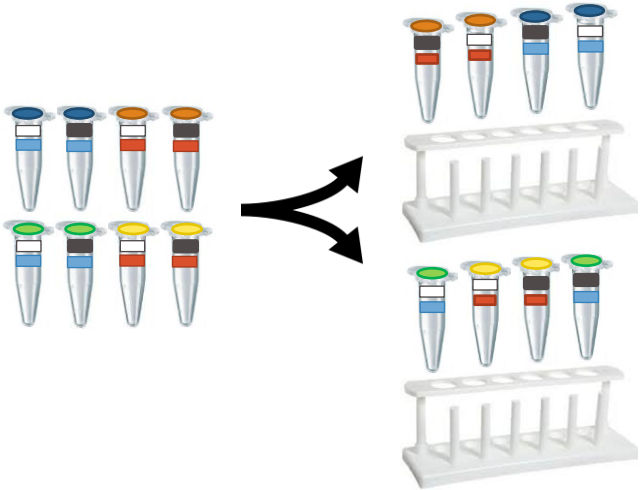

Plate	Females / Males	Time Points 1 / 2	Cases / Controls	N
1	4/0	2/2	2/2	4
2	0/4	2/2	2/2	4

D

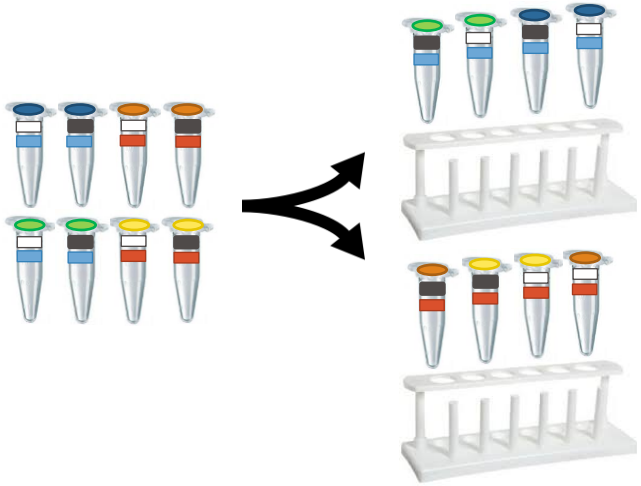

Plate	Females / Males	Time Points 1 / 2	Cases / Controls	N
1	2/2	2/2	4/0	4
2	2/2	2/2	0/4	4

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Checking randomization

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Cases vs Controls

E

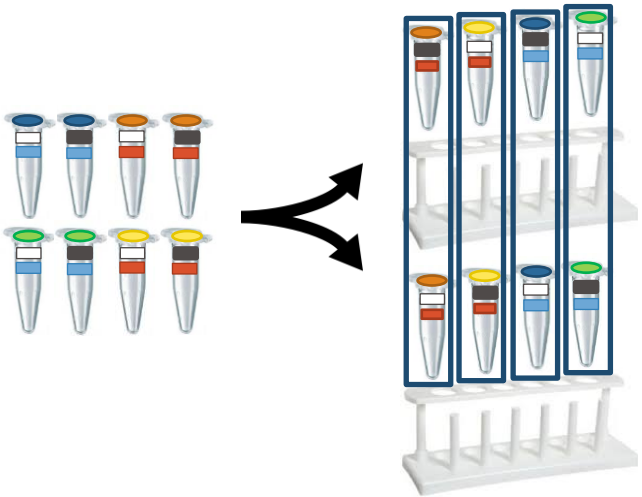

Plate	Females / Males	Time Points 1 / 2	Cases / Controls	N
1	2/2	2/2	2/2	4
2	2/2	2/2	2/2	4

F

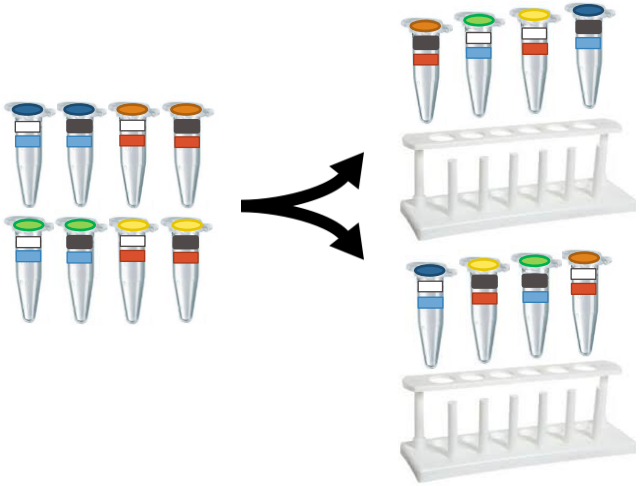

Plate	Females / Males	Time Points 1 / 2	Cases / Controls	N
1	2/2	2/2	2/2	4
2	2/2	2/2	2/2	4

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4. **Checking randomization in practice**
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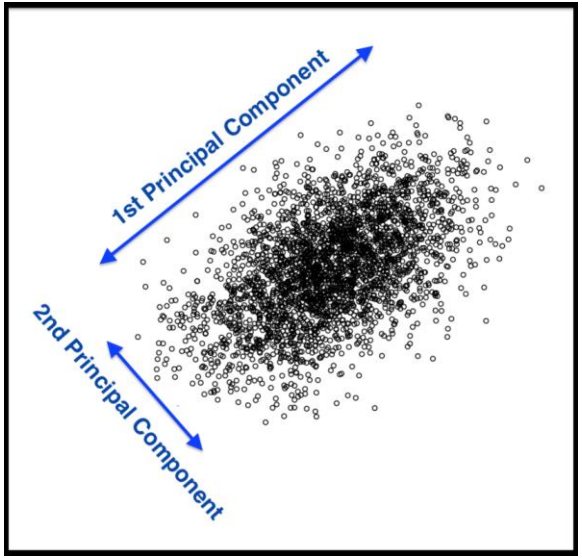
Checking randomization

- **Principal Component Analysis (PCA) plots**
- Predicting batches from additional variables
- Checking balance between and within batches
- Checking results by order of processing / user
- Checking measurement errors / variability by batch



Checking randomization

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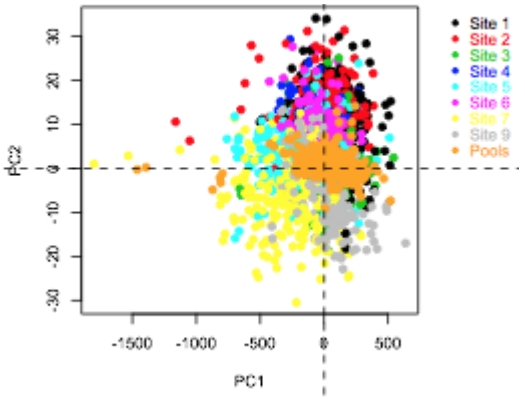
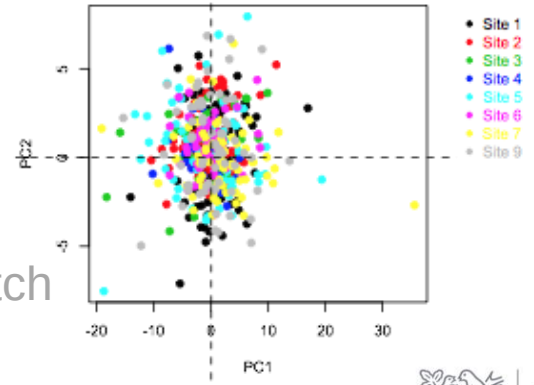
Batch effect



No batch effect



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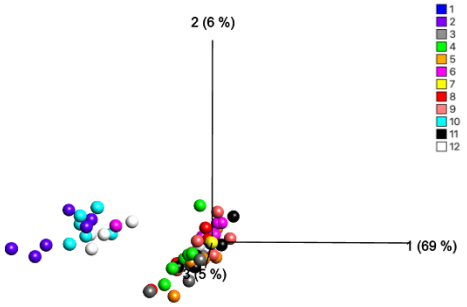

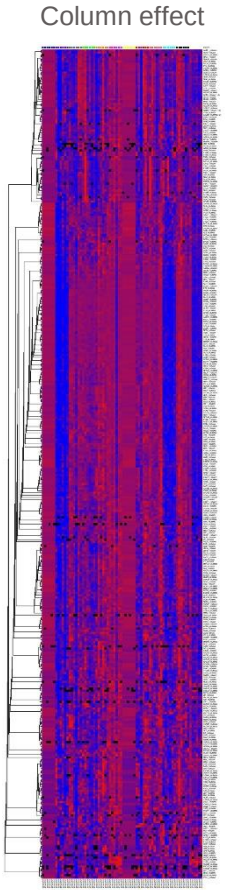

Checking randomization

- Principal Component Analysis (PCA) plots
- **Predicting batches from variables**
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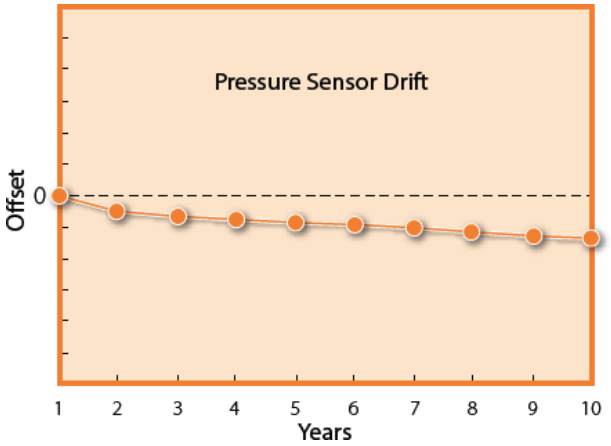
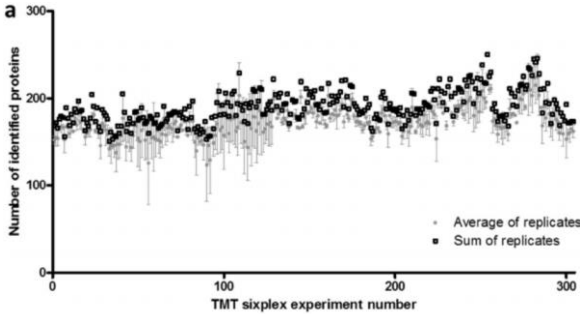


https://www.solinst.com/products/dataloggers-and-telemetry/3001-leve logger-series/technical-bulletins/understanding-pressure-sensor-drift.php



Cominetti et al. (2016) Proteomic Biomarker Discovery in 1000 Human Plasma Samples with Mass Spectrometry, *J. Proteome Res.* 2016, 15.

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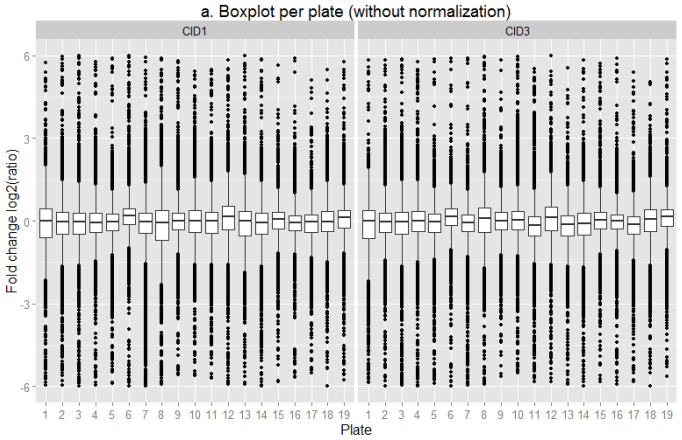

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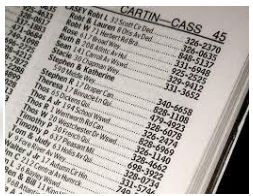
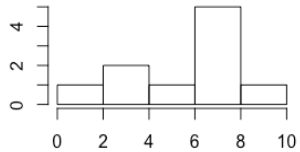
How do we use computers to randomize?

Computers use pseudo random number generators: sequence of numbers that are approximately random but are completely determined by an initial value: seed

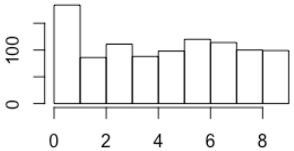
This allows reproducibility.

Low-tech random number generator:

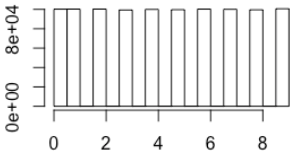




N = 10



N = 1'000



N = 1'000'000

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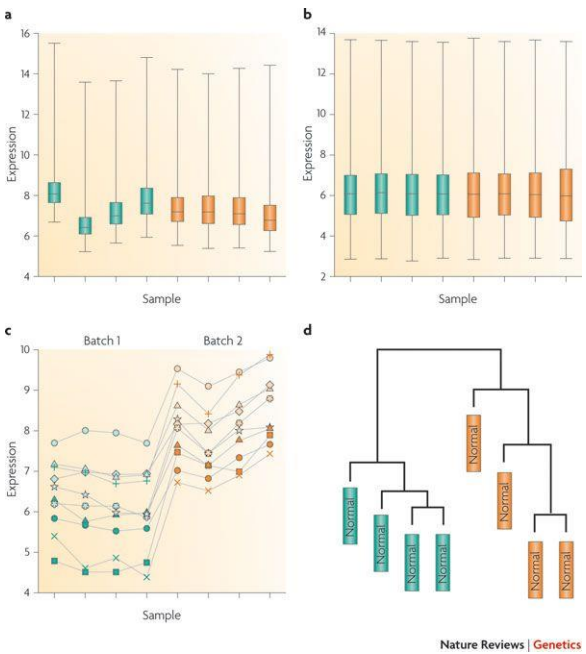
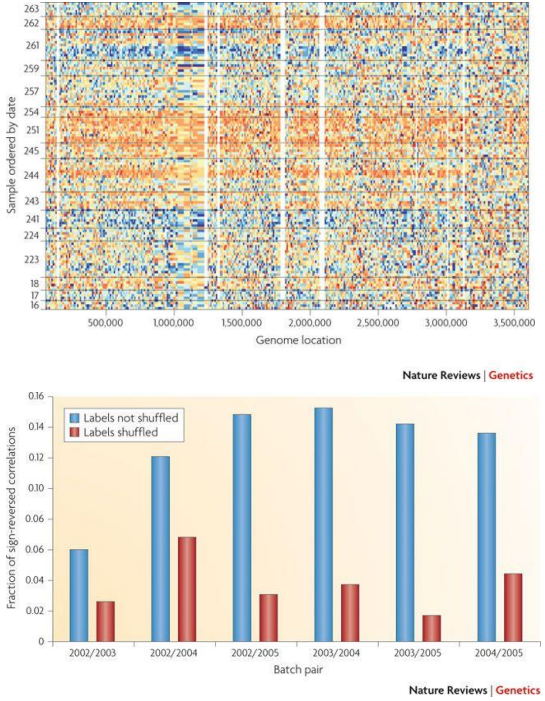
What can happen when randomization is not well-performed?





Leek et al. (2010) Tackling the widespread and critical impact of batch effects in high-throughput data, *Nature Reviews Genetics* volume11, pages733–739.

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Conclusions

- Randomization is very important, and it should be performed **before** running the analyses!

> “To consult the statistician after an experiment is finished is often merely to ask him to conduct a post mortem examination. He can perhaps say what the experiment died of.”
>
> Ronald Fisher

Conclusions

- Randomization is very important, and it should be performed **before** running the analyses!
- Randomization should be performed considering as many relevant variables as one can think of (date, site, gender, age, case/control, BMI, etc).
- After randomization has been performed, it should be checked that indeed batches are well randomized.
- Wish for the best! Even after randomizing there could still be still-unknown lurking (cachée / imprévue) variables that could invalidate the study and its conclusions.

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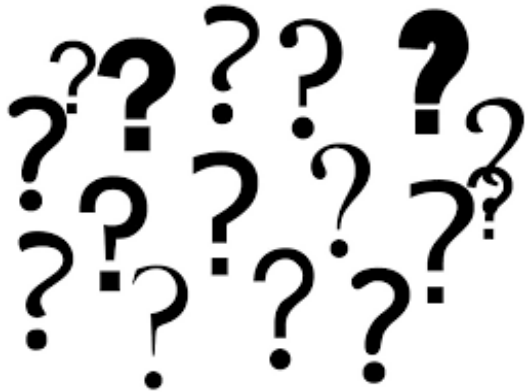
Questions?



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