Experimental design

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Introduction to Microarray technology course

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Experimental design...

- The purpose of the experimental design is to plan the experiment in a way that makes sure it can answer your biological question

- The experimental design is documented in the experimental plan

What is an experimental plan?

- A written document explaining the purpose of the experiment, and how it should be performed

- Think of the experimental plan as answering the old journalists requirements of 5 Ws and 1 H: who, what, when, where, why and how

Biological aspects (1)

- What is your hypothesis or question?
  - ✓ Does the samples reflect this?
- What else is known beforehand on the topic?
  - ✓ Expression levels on known interesting genes available?
- What samples are available?
  - ✓ More samples at a later time?
  - ✓ All biopsies shold be taken from the same part of the tissue!
  - ✓ Do you have enough RNA from each sample or is pooling of samples required?

Biological aspects (2)

- Choose the right model system

- Comparing the right things
  - ✓Does the cell type express the genes of interest?
  - ✓Make sure there are no confounding effects

Biological aspects (3)

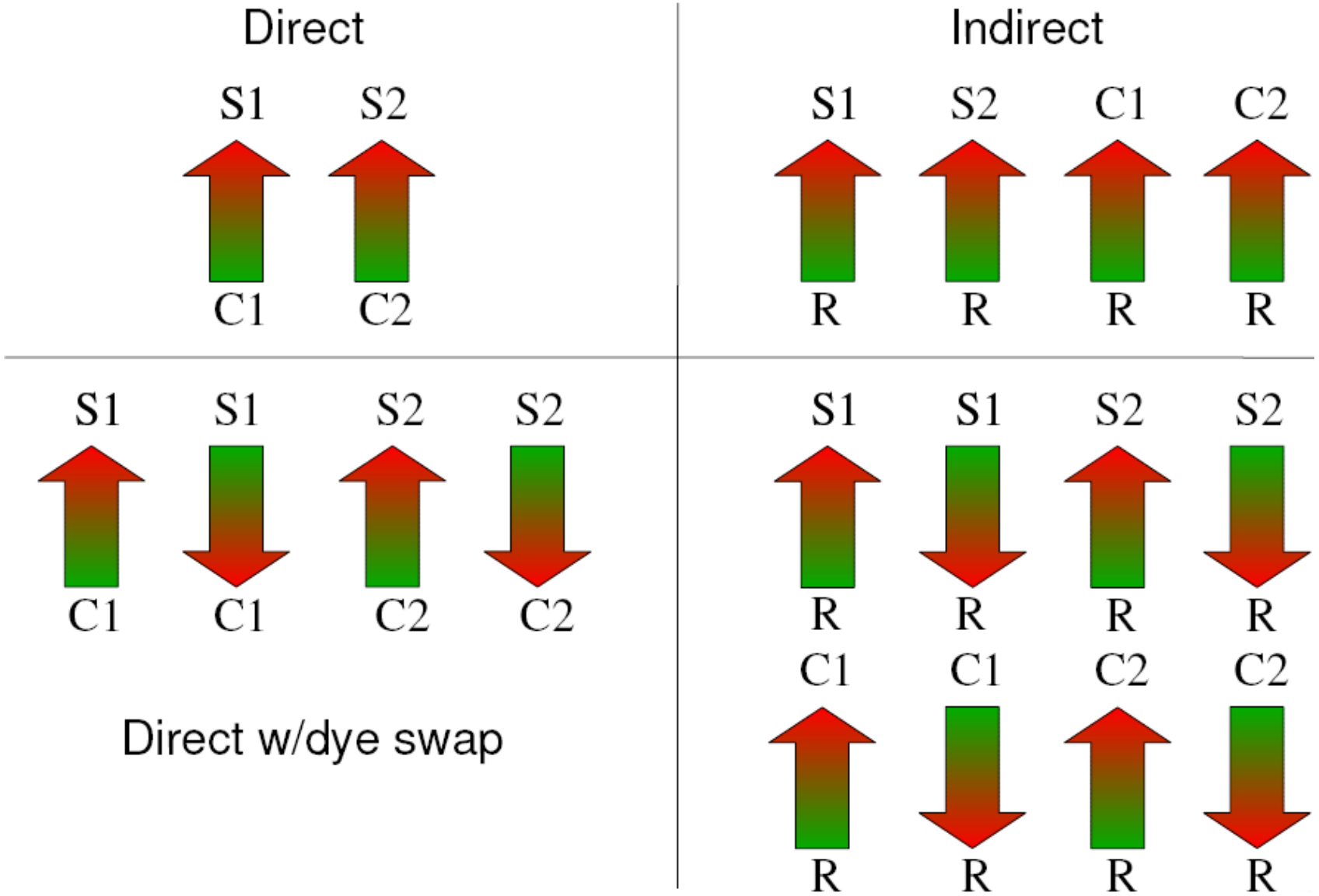
- Replicates
  - ✓ How large differences are you looking for?
  - ✓ What is the expected expression difference of targeted biology in these samples?
  - ✓ Will ”no change” be a desired significant result?
- Biological vs technical replicates
  - ✓ Use biologicl replicates to answer biological questions, and technical replicates to answer technical questions
  - ✓ What is a biological replicate?
    - Are cell cultures that originates from the same biological source biological or technical replicates?

Technical aspects (1)

- Choice of platform
  - ✓Closely linked to biological question
  - ✓Should be decided upon before doing the detailed planning of sampling and extraction
  - ✓One channel vs two channel?
    - One channel – one array – one sample
    - Two channel – one array – two samples
  - ✓Natural pairing between samples to be compared?
  - ✓Make sure key genes are on the array!

Technical aspects (2)

- Additional questions for two channel platform:
  - ✓ Are the samples naturally paired?
    - Before and after treatement?
  - ✓ Direct comparison vs indirect?
  - ✓ Dye swaps?
  - ✓ Common reference design
    - Representable, independent RNA
    - Dye swaps strongly recommended
    - More noisy
    - More flexible



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Technical aspects (3)

- Aviod systematic errors
  - ✓ Technical noise added to experiment at each step
  - ✓ Ideal: each step, one person, one protocol one day
  - ✓ Important to identify these steps in order to be able to control them
    - Know your batches!
  - ✓ Sample groups should be balanced across the batches
  - ✓ Randomize the order of treatement within a batch
  - ✓ Use the same batch of arrays and reagents within an experiment
- Plan biological replicates
  - ✓ Statistical significance of findings
  - ✓ Choose results to validate

Physical aspects

- Limitations to the equipment
  - ✓What is the limiting point?
- Get to know your bathces
  - ✓How many samples are possible to do at the same time at the different steps?
- How many people are/should be involved?
  - ✓Who does what?

Economical aspects

- What is the budget?
  - ✓ Experimental costs
  - ✓ Analysis costs
  - ✓ Time costs!

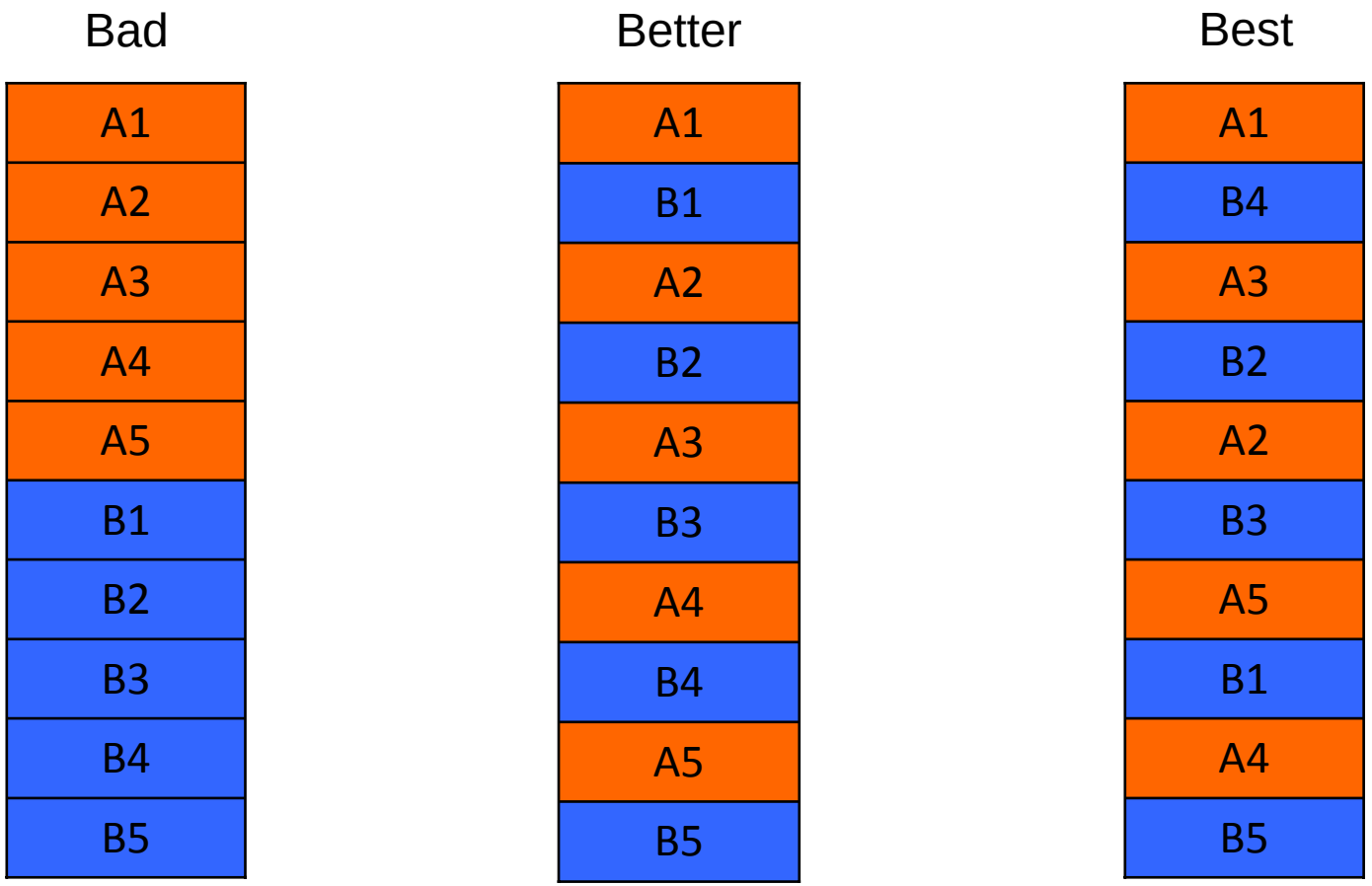
- Pilot necessary?
  - ✓ Plan to balance batch effect if pilot runs shoud be used in final study

- Prioritize some groups/contrasts, and run more biological replicates?

General strategy

- For each step to control for systematic bias:
  - ✓ Distribute the biological groups systematically in a balanced fashion: for two groups of equal size, every second from each group
  - ✓ Divide it into roughly equal size batches limited by your capacity for the step
- Tip: In excel (or similar program) colour code the sample names by biological group and the column next to it by batch
- Randomization and balancing with respect to biology of interest: Possible to separate technical variation from biological variation

How to order



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Experimental plan: an example

Biology
A1
A2
A3
A4
A5
A6
B1
B2
B3
B4
B5
B6
C1
C2
C3
C4
C5
C6

Biology	Sampling order
A1	1
B4	2
C2	3
A3	4
B6	5
C4	6
A5	7
B2	8
C6	9
A2	10
B3	11
C1	12
A4	13
B5	14
C3	15
A6	16
B1	17
C5	18

Biology	Sampling order	Extraction order
A2	10	1
B6	5	2
C1	12	3
A5	7	4
B5	14	5
C6	9	6
A6	16	7
B4	2	8
C5	18	9
A3	4	10
C3	15	11
B2	8	12
A4	13	13
C4	6	14
B1	17	15
A1	1	16
B3	11	17
C2	3	18

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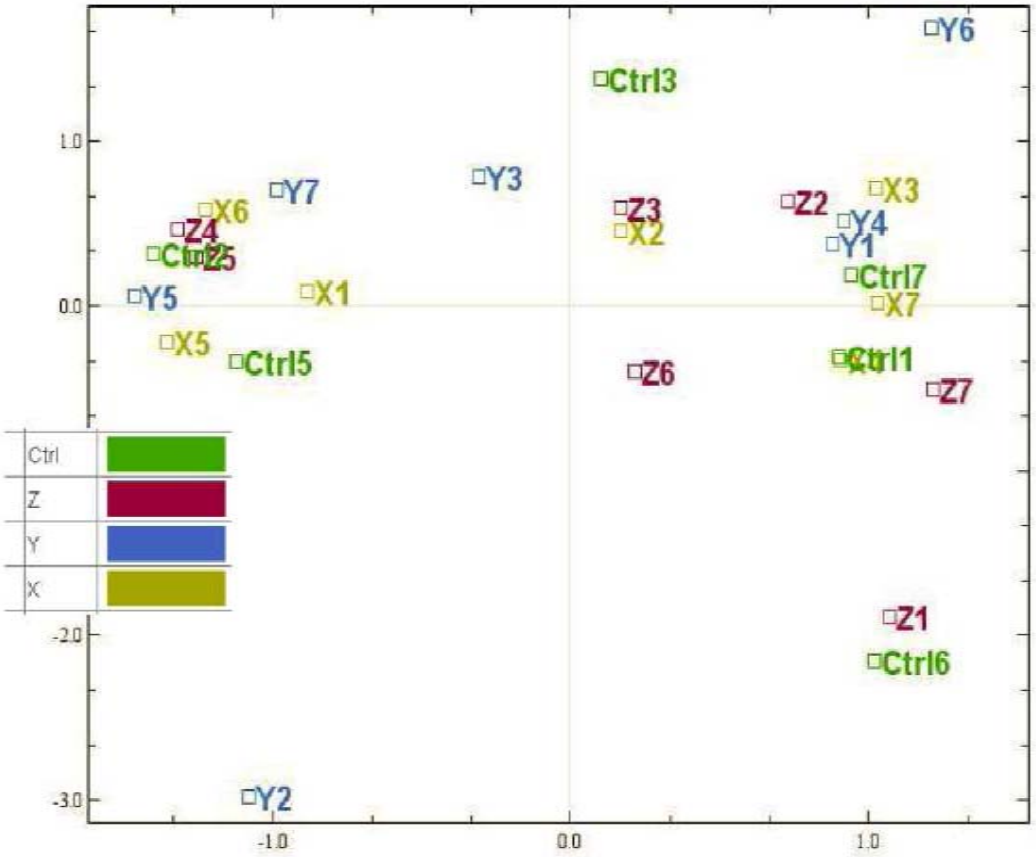
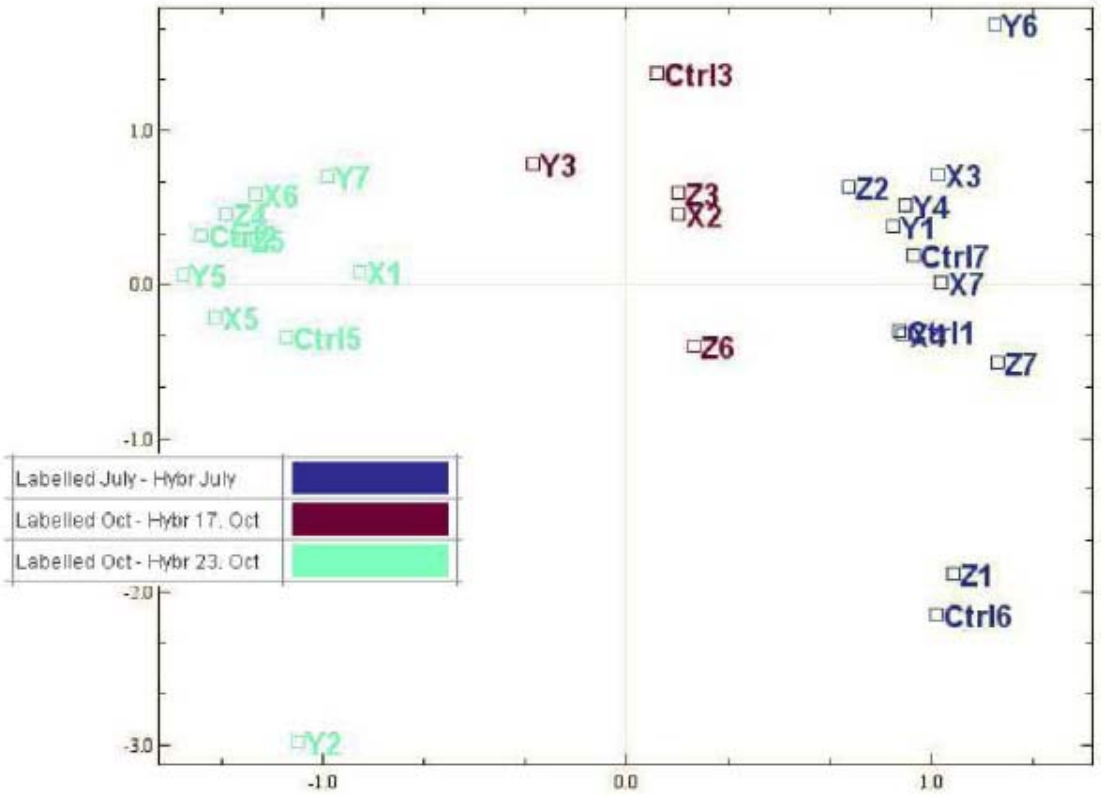
Batch effect: an example (1)



Samples labeled according to biology

Batch effect: an example (2)



Samples labeled according to labelling date

Summary

- Plan ahead!
- Randomization and balancing
- Write it down in an Experimental plan
- Follow the experimental plan!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Practical exercise 1

- You have 16 animals, 8 treated (T1-8) and 8 controls (C1-8)
- Your sampling capacity is 5 animals per day
- In what order will you do the sampling?

Practical exercise 2

- 16 samples, 8 treated(T1-T8), 8 controls (C1-C8)
- Can extract 12 samples at the same time because of centrifuge capacity

- How would you organize the extraction?

Practical exercise 3

- The same samples will be hybridized to the arrays, one samle per array
- You have 20 arrays available, 12 from batch A, 8 from batch B
- How will you assign the samples to arrays?