

Experimental Setup and Design

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Experimental Setup and Design

- Biological aspects
 - What is your hypothesis or question?
 - What else is known beforehand on the topic ?
- Technical aspects
 - Compare the right things to each other
 - Choice of platform
 - Avoid systematic errors
 - Ideal: each step, one person, one protocol, one day
 - Plan biological replicates
 - statistical significance of findings
 - choose results to validate
- What is the budget
 - Experimental and analysis costs



Comparing the right things

- Make sure there are no confounding effects
 - E.g. samples from patients vs control where all patients are old and all controls are young
- Biopsies should be taken from the same part of tissue
 - E.g. If one sample cuts through a major vein, differences seen could be due to different cell type compositions
- Chose the right model system
 - Do the cell types present express the genes of interest, e.g. not all treatments can be evaluated by blood samples, or in cell cultures



Choice of platform

- Choice of platform is closely linked to the biological question and has some implications on the rest of the design, so should be decided upon before doing the detailed planning of sampling and RNA extraction.
 - One channel vs two channel?
 - Natural pairing between samples to be compared?
 - Make sure key genes are on the array



Platform independent questions

- What is the difference you want to measure?
 - Do the samples reflect this ?
- Do we have to pool RNA to get enough for hybridisation?
- Do the arrays have spike probes and do we want to use them?

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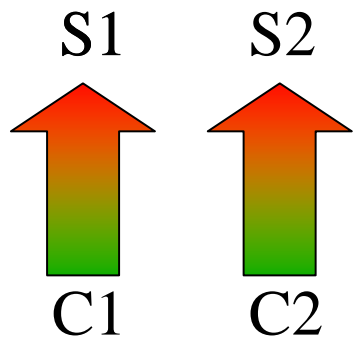


Additional questions for two channel platforms

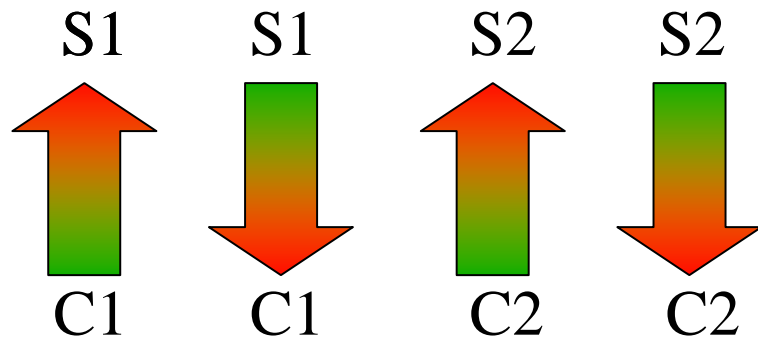
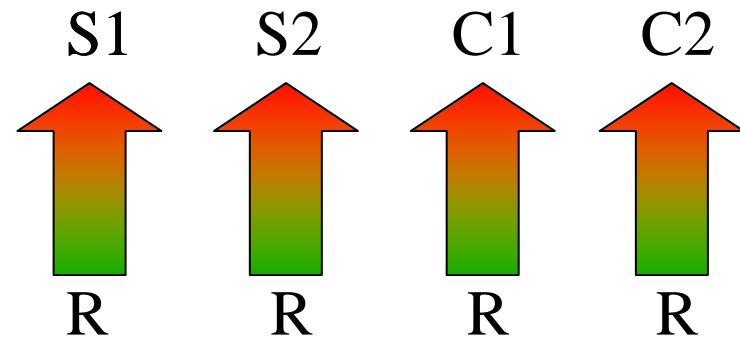
- Are the samples naturally paired ?
 - Before and after treatment
- Direct comparison vs indirect ?
- Dye swaps ?
- Common reference design
 - Representable, independent RNA
 - Dye swaps strongly recommended
 - More noisy
 - More flexible



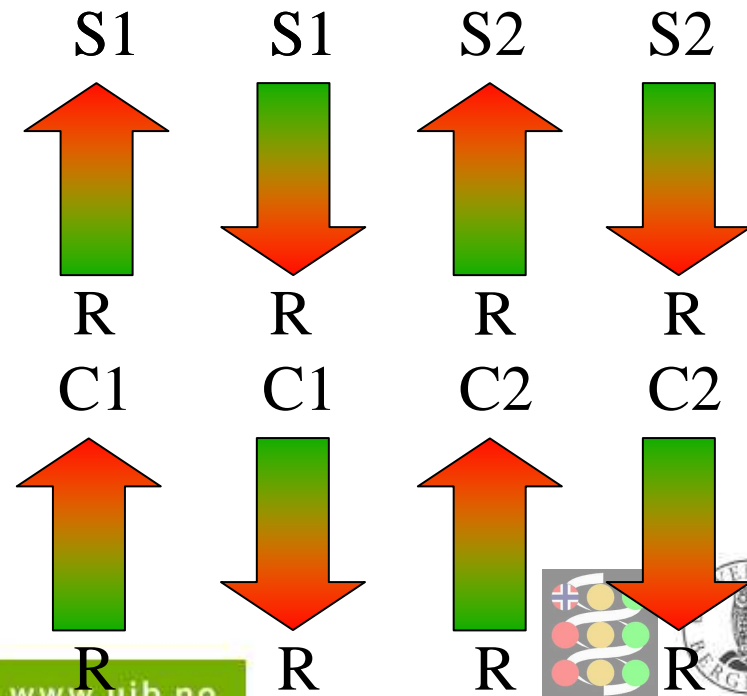
Direct



Indirect



Direct w/dye swap



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Avoid systematic errors

- Technical noise is added to the experiment every time you touch a sample at any step. It is important to control this, so that equal amounts of noise is added to all samples across the sample groups
- Common steps to control, include sampling, RNA extraction, labeling and hybridisation, but there may be others in your particular experiment
- Important to identify all steps that can add noise and that can be controlled.
- To control the steps you must also get to know your batches
 - Capacity per day
 - Persons involved
- The sample groups should generally balance across the batches
- Randomize the order of treatment within a batch



How to order

Bad

A1
A2
A3
A4
A5
B1
B2
B3
B4
B5

Better

A1
B1
A2
B2
A3
B3
A4
B4
A5
B5

Best

A1
B4
A3
B2
A2
B3
A5
B1
A4
B5

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General strategy

- For each step to control for systematic bias:
 - Distribute the biologic groups systematically in a balanced fashion: for two groups of equal size, every second from each group
 - Within each biological group, reshuffle the order of these samples
 - Divide it into roughly equal same size batches limited by your capacity for the step
- Tip: Colour code the sample names by biological group and the column next to it by batch



Experiment plan example

Biology	Sampling order	Extraction order
A1	A1	A4
A2	B4	B3
A3	A3	A5
A4	B2	B4
A5	A2	A1
B1	B3	B1
B2	A5	A3
B3	B1	B5
B4	A4	A2
B5	B5	B2

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Exp plan with batches

Biology	Sampling order	Extraction order
A1	A1	A4
A2	B4	B3
A3	A3	A5
A4	B2	B4
A5	A2	A1
B1	B3	B1
B2	A5	A3
B3	B1	B5
B4	A4	A2
B5	B5	B2

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How many replicates?

- How many do you have ready now?
 - Will you get more later?
- 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 ?

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Biological vs technical replicates

- Generally we use biological 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?
- What biological generality of your results are you aiming for?
- What is your long term plan ? Except for getting things published...

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Budget

- Pilot necessary?
 - Plan to balance batch effect if pilot runs should be used in final study
- Prioritize some groups/contrasts, and run more biological replicates ?
- Time is money also in academia, remember analysis costs.

