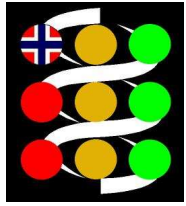


Experimental Setup and Design

Kjell Petersen

Microarray.no

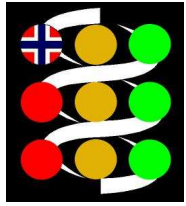




Experimental Setup and Design



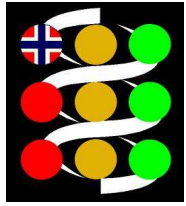
- Biological aspects
 - ✓ What is your hypothesis or question?
 - ✓ What else is known beforehand on the topic ?
- Technical aspects
 - ✓ Compare the right things to each other
 - ✓ Avoid systematic errors
 - Ideal: each step, one person, one protocol, one day
 - ✓ Plan biological replicates
 - statistical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



Experimental Design



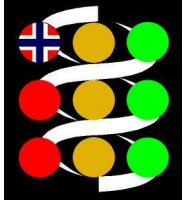
- Hybridize what to what?
 - ✓ Compare the right things
 - ✓ i.e. what is your question?
- Avoid systematic errors
 - ✓ Plan the order of how samples are processed
- Plan the statistics
 - ✓ How many replicates?
- What is the budget
 - ✓ Experimental and analysis costs



Hybridize what to what?



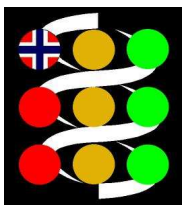
- 1 channel: simple case?
 - ✓ Preparation of RNA
 - ✓ Spike-ins
 - ✓ Pools ?
- What is the difference you want to measure?
 - ✓ Do the samples reflect this ?



Hybridize what to what cont.

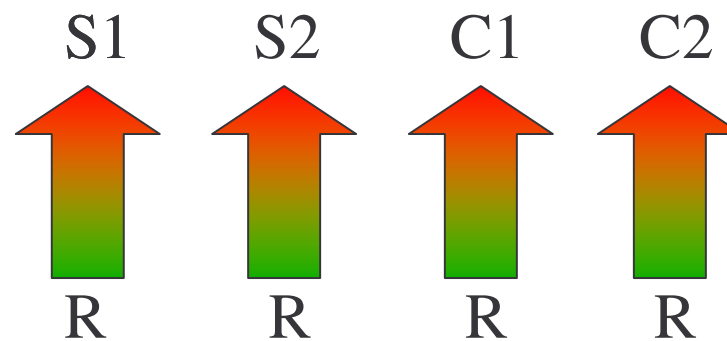


- 2 channel platform, additional questions:
 - ✓ Are the samples naturally paired ?
 - Before and after treatment
 - ✓ Direct comparison vs indirect ?
 - ✓ Dye swaps ?
- Common reference design
 - ✓ Representable, independent RNA
 - ✓ Dye swaps strongly recommended
 - ✓ More noisy
 - ✓ More flexible

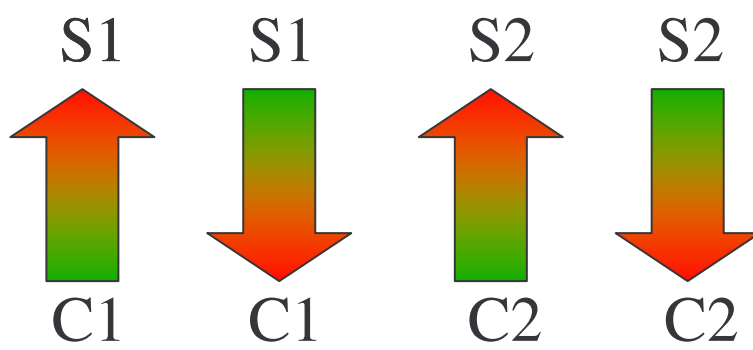


Direct

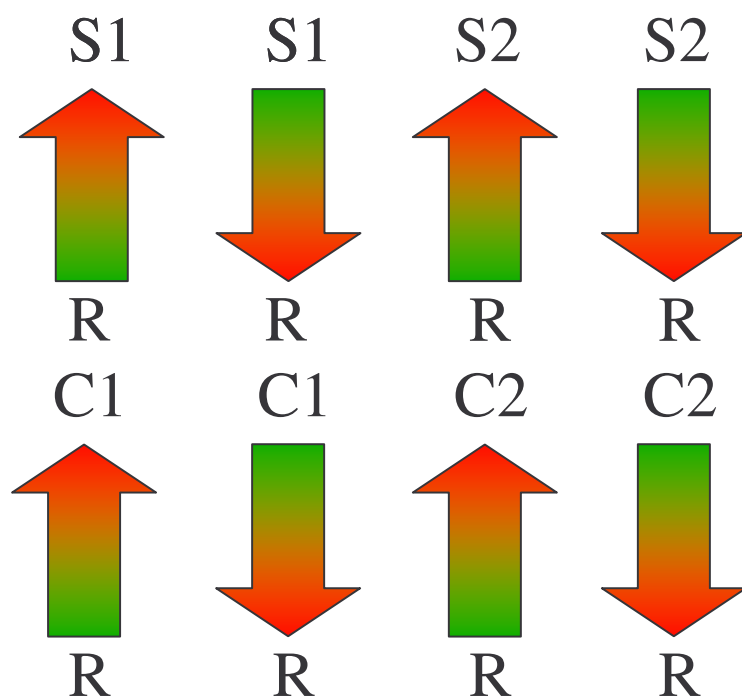
Indirect

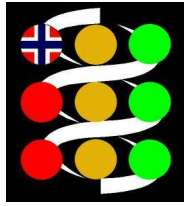


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Direct w/dye swap

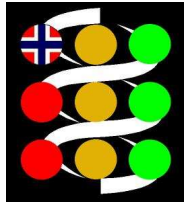




Avoid systematic errors



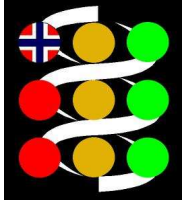
- Get to know your batches
 - ✓ Capacity per day
 - ✓ Persons involved
 - ✓ Equal size batches
- The sample groups should generally balance across the batches
- Randomize the order of treatment within a batch



General strategy



- For each step to control for systematic bias:
 - Make a random sequence of numbers representing the random draw of samples
 - Sort the samples by their order
 - Divide it into roughly equal same size batches limited by your capacity for the step
 - Swap order of individual pairs of samples to improve sample group balance across batches
- Tip: Colour code the sample names by group and the sample order by batch



How to order



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Bad

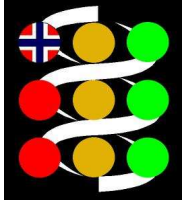
A1
A2
A3
A4
A5
B1
B2
B3
B4
B5

Better

A1
B1
A2
B2
A3
B3
A4
B4
A5
B5

Best

A1
B4
A3
B2
A2
B3
A5
B1
A4
B5

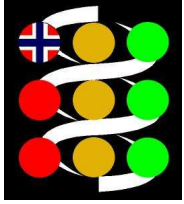


Experiment plan example



Microarray.no

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

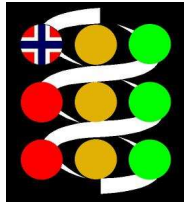


Exp plan with batches



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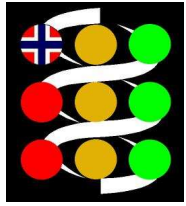
Biology	Sampling order	Extraction order
A1	A1	A4
A2	B4	B3
A3	A3	A5
A4	B2	B4
A5	A2	A1
B1	B3	B1
B2	A5	A3
B3	B1	B5
B4	A4	A2
B5	B5	B2



How many replicates?



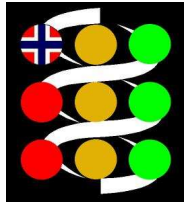
- How many do you have ready now?
 - ✓ Will you get more later?
- How large differences are you looking for?
- What is the expected expression difference of targeted biology in these samples ?
- Will “no change” be a desired significant result ?



• Biological vs technical repl



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



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.