Introduction to Experimental Design

Chapter 2

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KEY PRINCIPLES in Experimental Design

- Controls
- Replication
- Blocking
- Randomisation
- Blinding

KEY PRINCIPLES in Experimental Design

- Controls
  - Direct comparison with a known standard or no treatment.
  - Tested under identical conditions to experimental treatment.
- Replication
- Blocking
- Randomisation
- Blinding

KEY PRINCIPLES in Experimental Design

- Controls
- Replication repeating experiment on different samples to:
  1. Increase precision of treatment effect
  2. Make result more generalisable
- Blocking
- Randomisation
- Blinding

KEY PRINCIPLES in Experimental Design

- Controls
- Replication
- Blocking
  - Grouping together similar experimental units
  - Comparing treatments within homogeneous groups
- Randomisation
- Blinding

KEY PRINCIPLES in Experimental Design

- Controls
- Replication
- Blocking
- Randomisation
  - probabilistic process of assigning treatment
  - randomising order of testing
- Blinding

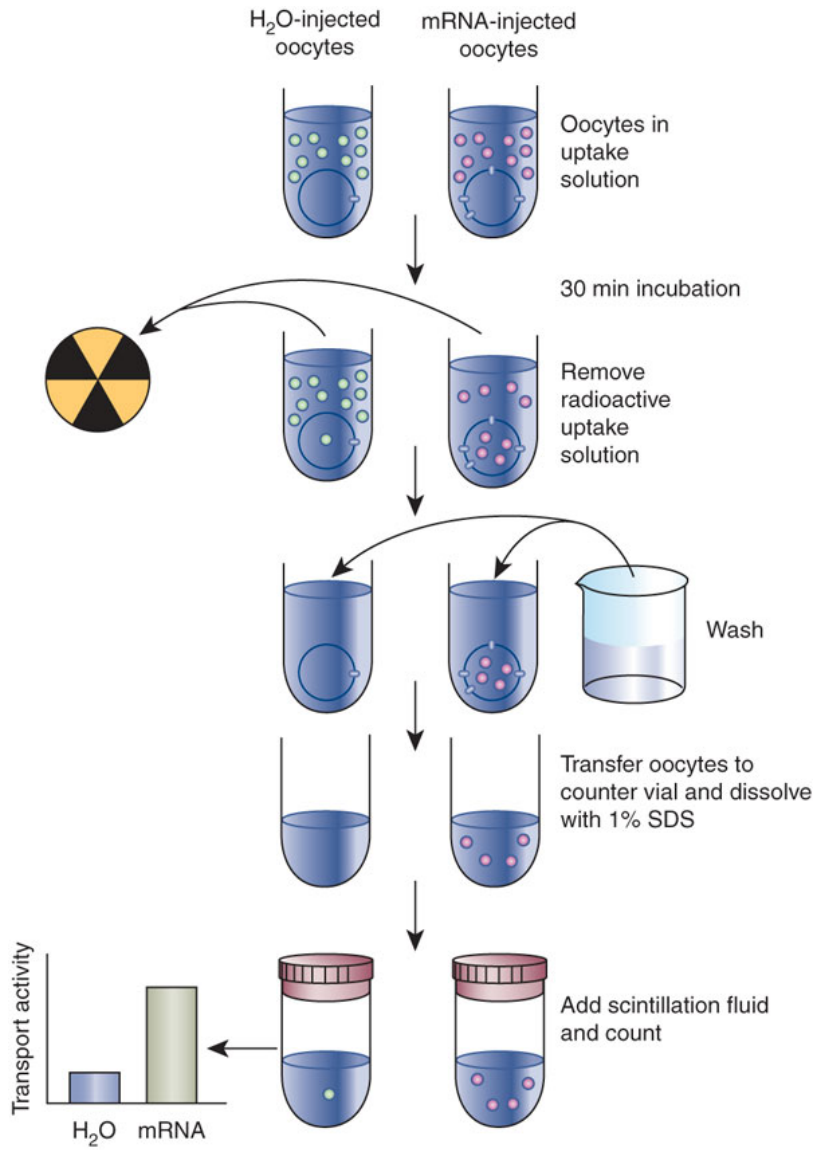
KEY PRINCIPLES in Experimental Design

- Controls
- Replication
- Blocking
- Randomisation
- Blinding Masking treatment assignment
  - Allocation blinded
  - Evaluator blinded

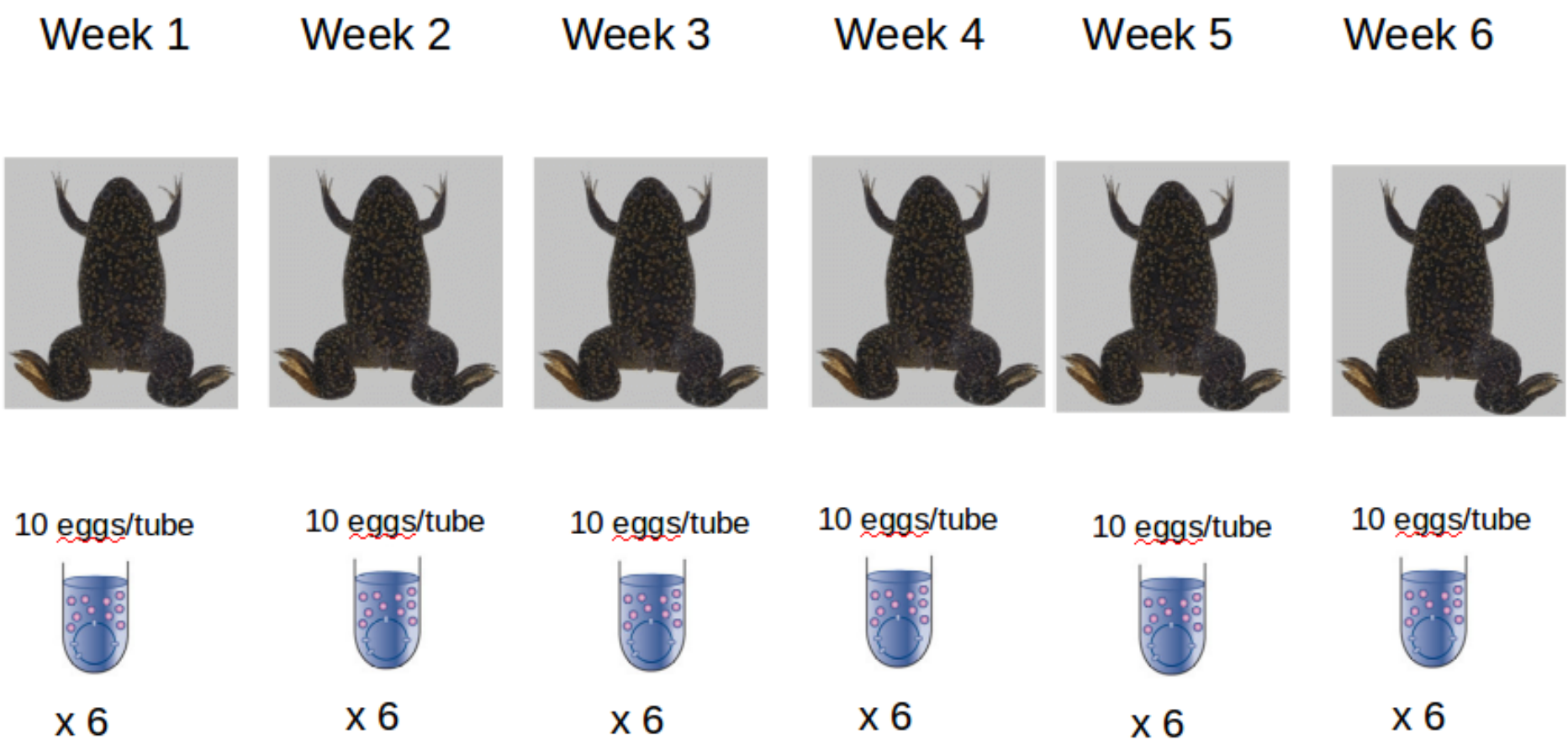
Example: Converging on an experimental design using these key principles

Research context:

- What are the essential elements of chloroquine transporters in malaria parasites?
- Methods: oocyte system, radiotracer assay
- Treatments: 5 mutant transporters plus wild type



Experimental design: chloroquine transporters



Experimental design: chloroquine transporters

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Wild-type	Mutant 1	Mutant 2	Mutant 3	Mutant 4	Mutant 5
					
10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube
					
x 6	x 6	x 6	x 6	x 6	x 6

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What is wrong with this design?

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- CONTROLS: not tested under identical conditions
- REPLICATION: only pseudo-replication
- BLOCKING: none
- RANDOMISATION: NA

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- CONTROLS: not tested under identical conditions
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- BLOCKING: none
- RANDOMISATION: NA

Experiment is useless

How about this design

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Wild-type Mutant 1	Wild-type Mutant 2	Wild-type Mutant 3	Wild-type Mutant 4	Wild-type Mutant 5	Mutant 1 Mutant 2
					
10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube
					
x 6	x 6	x 6	x 6	x 6	x 6

How about this design

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Wild-type Mutant 1	Wild-type Mutant 2	Wild-type Mutant 3	Wild-type Mutant 4	Wild-type Mutant 5	Mutant 1 Mutant 2
					
10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube	10 eggs/tube
					
x 6	x 6	x 6	x 6	x 6	x 6

What is wrong with this design?

What is wrong with this design?

- CONTROLS: just okay, can compare control to each mutant; not between mutants
- BLOCKING: frog=block, not all treatments for each block
- REPLICATION: no replication of any comparison
- RANDOMISATION: could randomise tubes within day

Also, half the eggs were used for control treatment: is this the most efficient use of resources?

How about this design

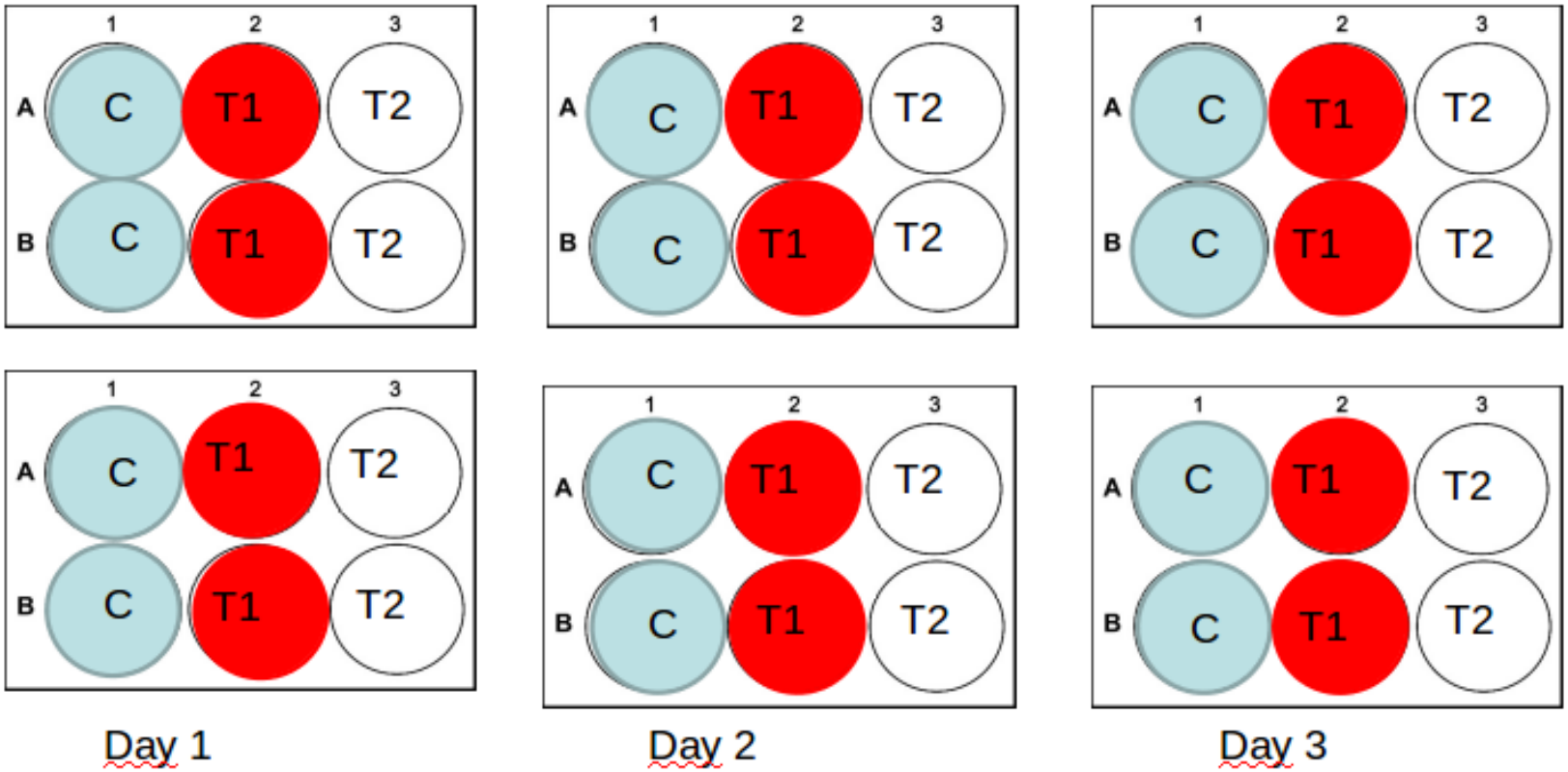
Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Wild-type	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type
Mutant 1	Mutant 1	Mutant 1	Mutant 1	Mutant 1	Mutant 1
Mutant 2	Mutant 2	Mutant 2	Mutant 2	Mutant 2	Mutant 2
…	…	…	…	…	…
Mutant 5	Mutant 5	Mutant 5	Mutant 5	Mutant 5	Mutant 5
5 eggs/tube	5 eggs/tube	5 eggs/tube	5 eggs/tube	5 eggs/tube	5 eggs/tube
					
x 12	x 12	x 12	x 12	x 12	x 12

A good design

- CONTROLS: yes, can compare control to each mutant, and each mutant to every other
- BLOCKING: frog=block, complete randomised design
- REPLICATION: each block is a replicate
- RANDOMISATION: could randomise tubes within day

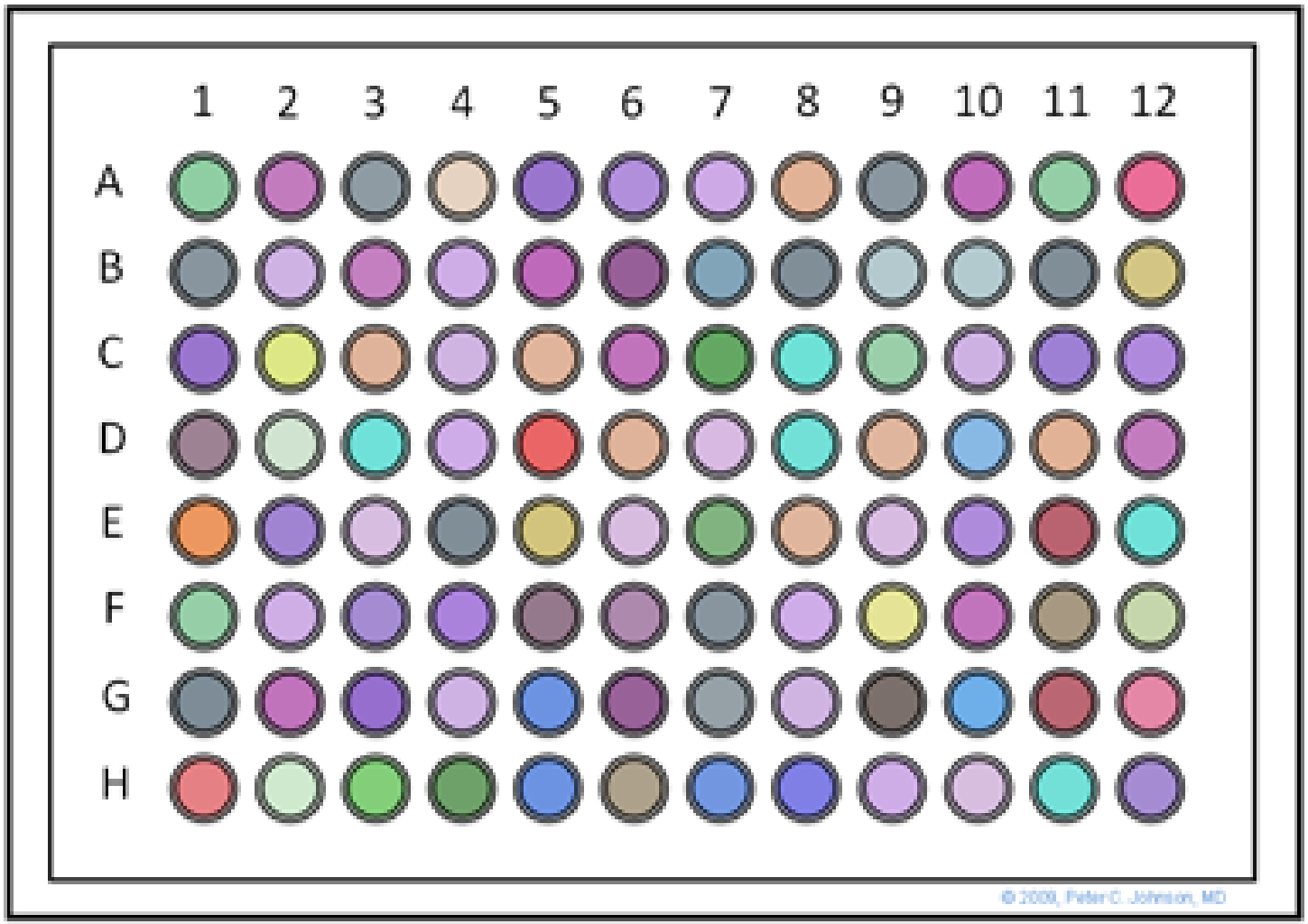
Example 2

How can the design of this experiment (control + 2 treatments) be improved?



Example 3

What are the possible sources of variation on a plate for a PCR / cell viability plate / plant experiment



Example 4

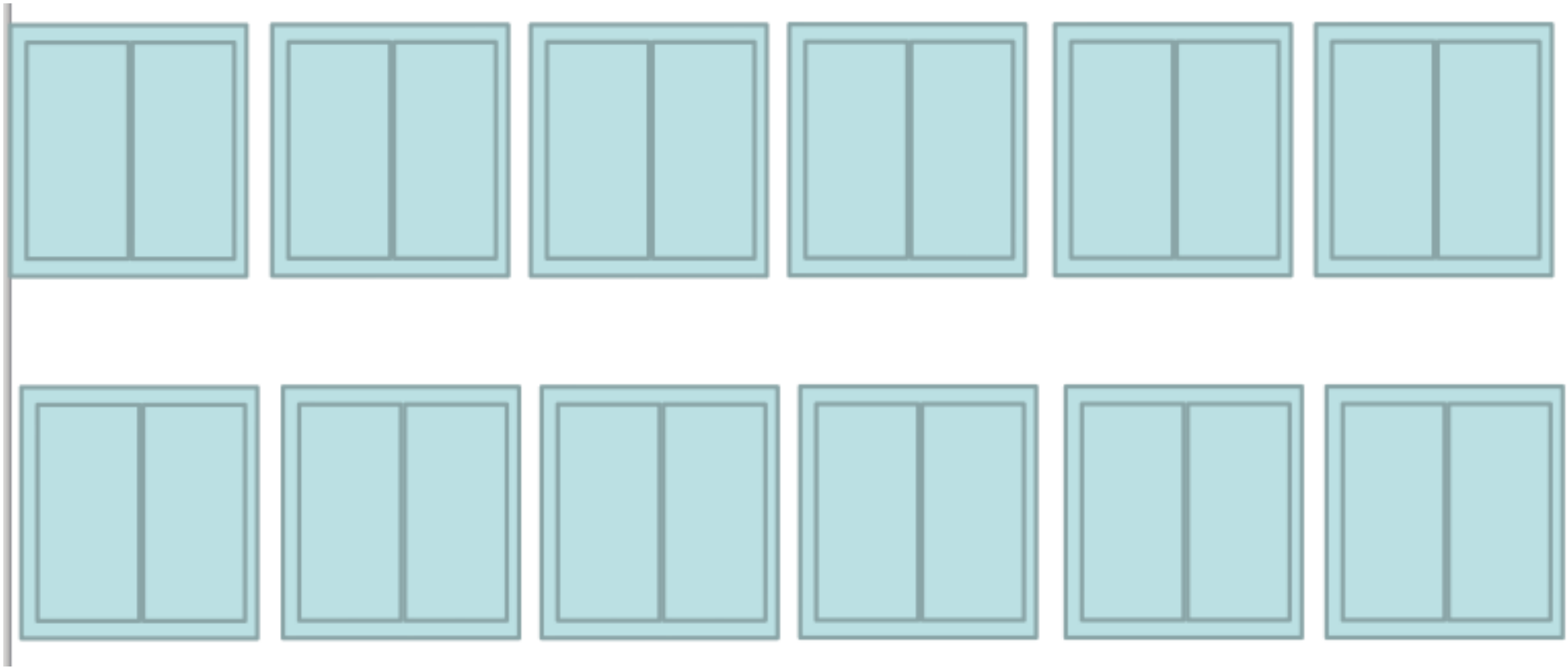
Cochrane et al. Oikos (2014)

Research context

- How is seedling emergence (in Banksia) influenced by temperature and moisture?
- Set up: 12 shelters, 2 garden beds per shelter, 24 pots per bed.
- Experimental factors:
  - Temperature (2 levels)
  - Water (3 levels)
  - Species (4 levels)
  - Populations (6 per species = 24)

Example 4

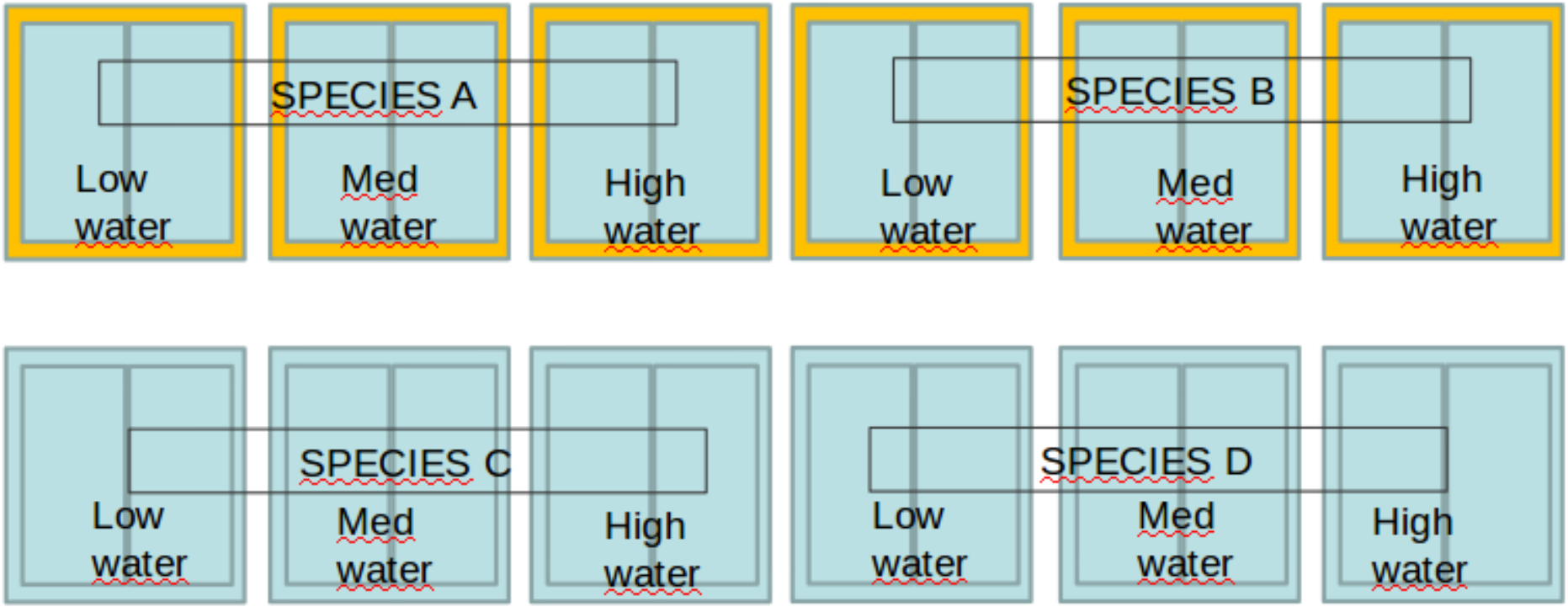
How to distribute treatments across 12 shelters?



Example 4

How to distribute treatments across 12 shelters?

Hot temperature in orange, cold in turquoise



Example 4

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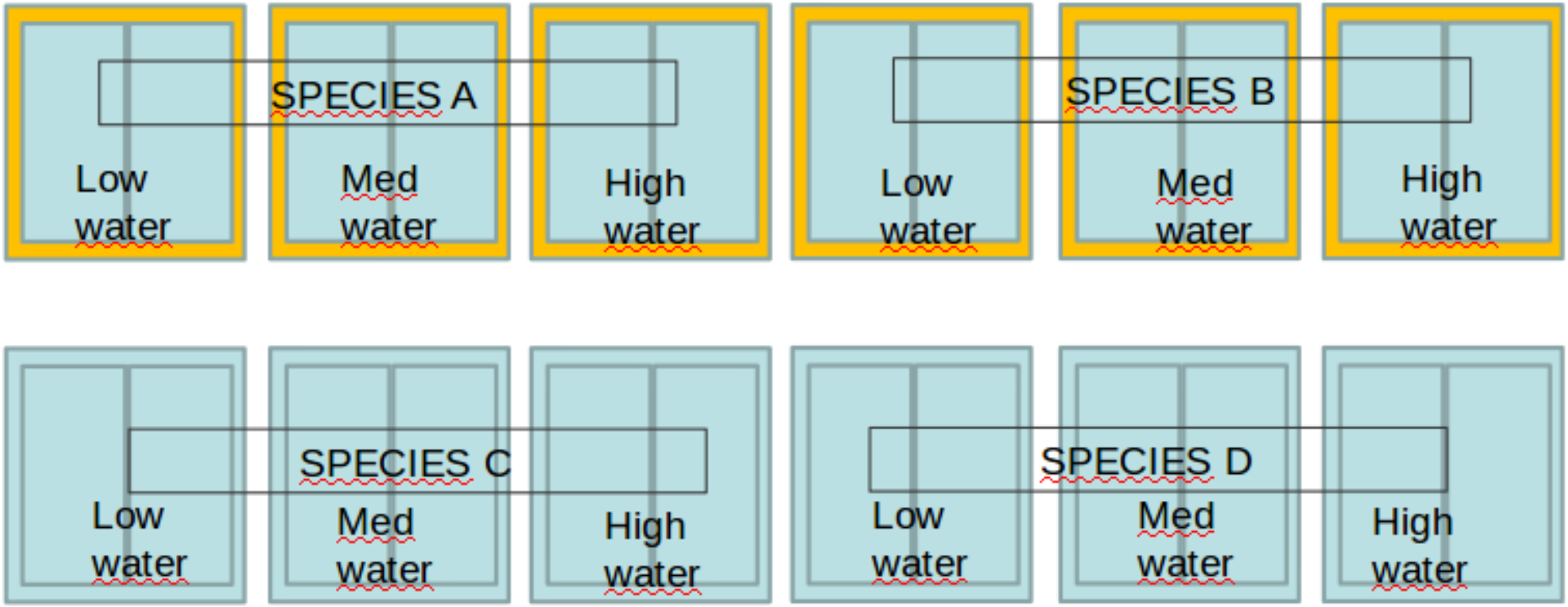
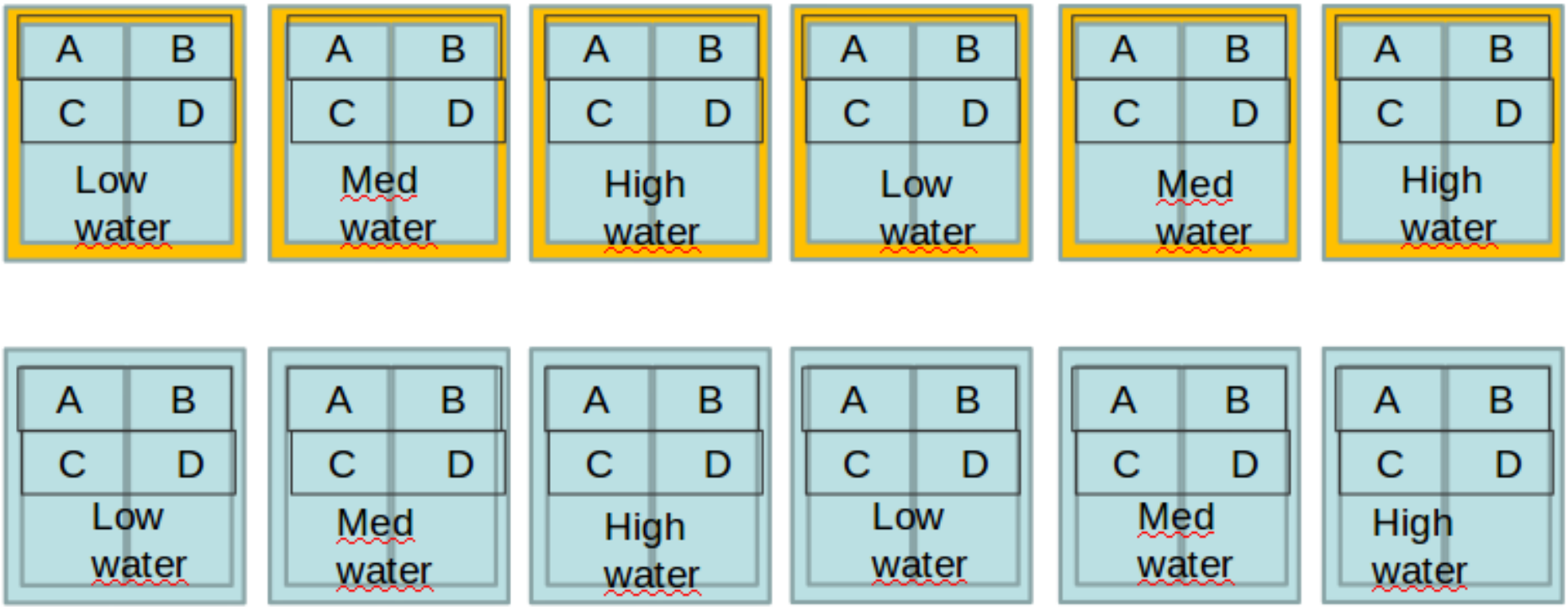


What's wrong with this design?

Example 4

How to distribute treatments across 12 shelters?
How about this design?

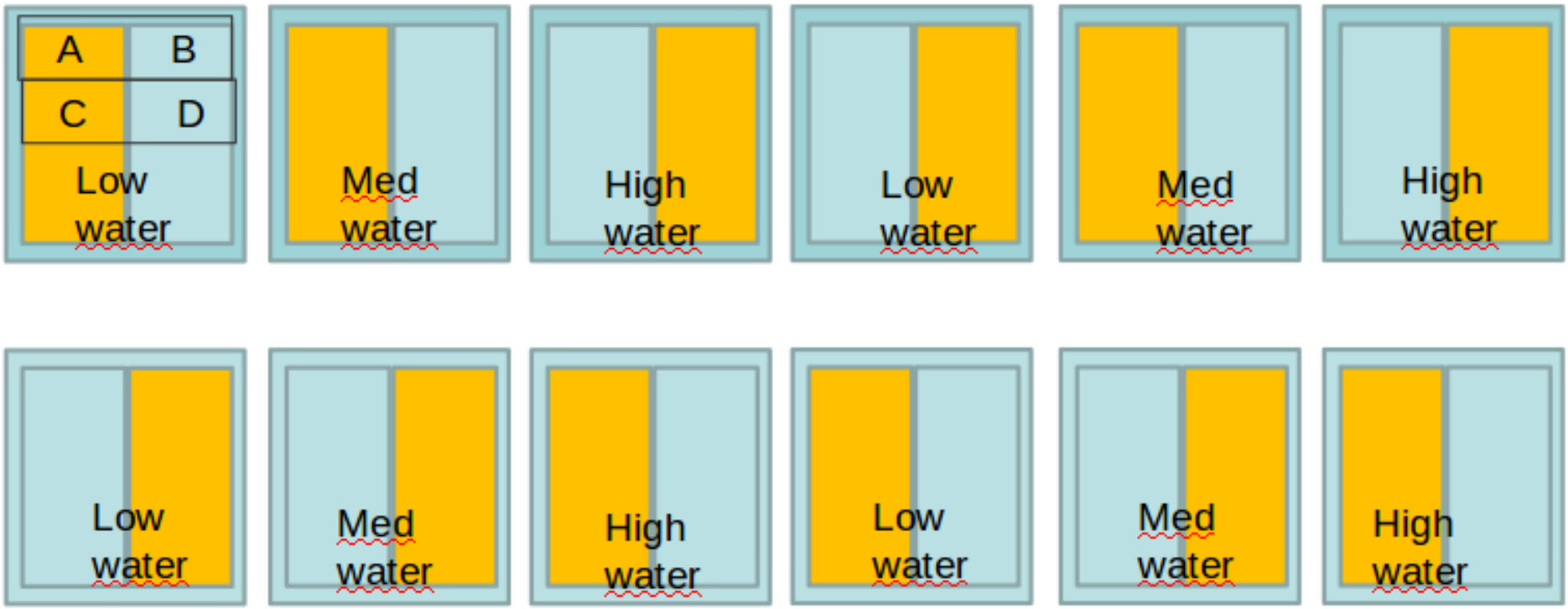
- Hot temperature in top shelters, cold in bottom shelters
- repeat A/B/C/D in each shelter



Example 4

How to distribute treatments across 12 shelters?
How about this design?

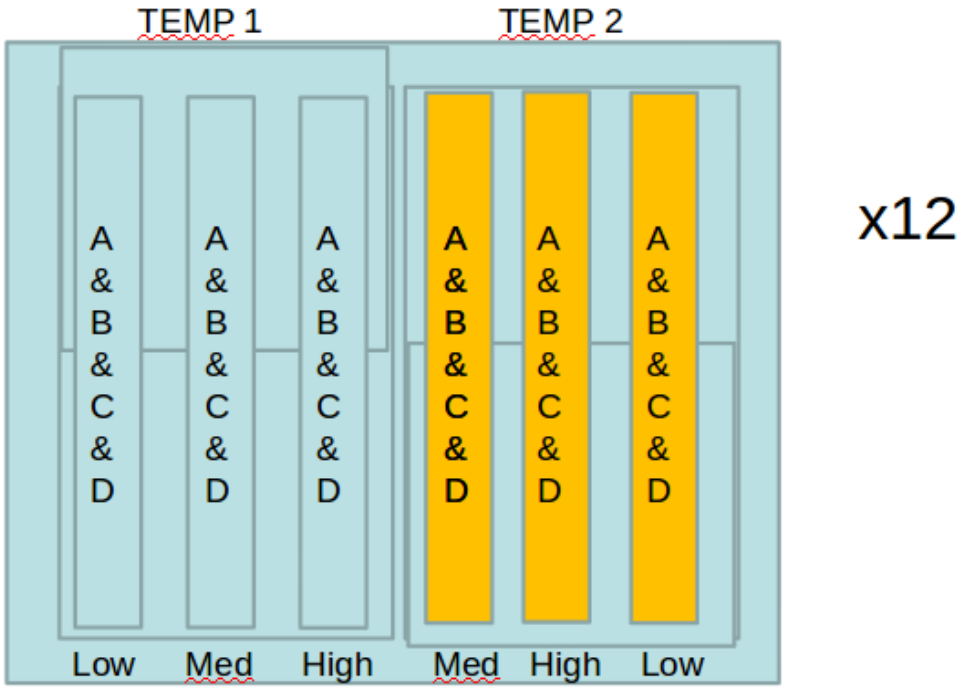
- Hot and cold temperatures by bed (randomized left/right)
- repeat A/B/C/D in each shelter



Example 4

How to distribute treatments across 12 shelters?
How about this design?

- Hot and cold temperatures by bed (randomized left/right)
- repeat A/B/C/D in each row (randomized within row)
- Humidity in each bed (randomized among columns)



x12

Reading

- Chapter 3, Statistical methods in biology, Welham et al
- Kilkenny et al ARRIVE guidelines
- Ten Simple Rules for Effective Statistical Practice