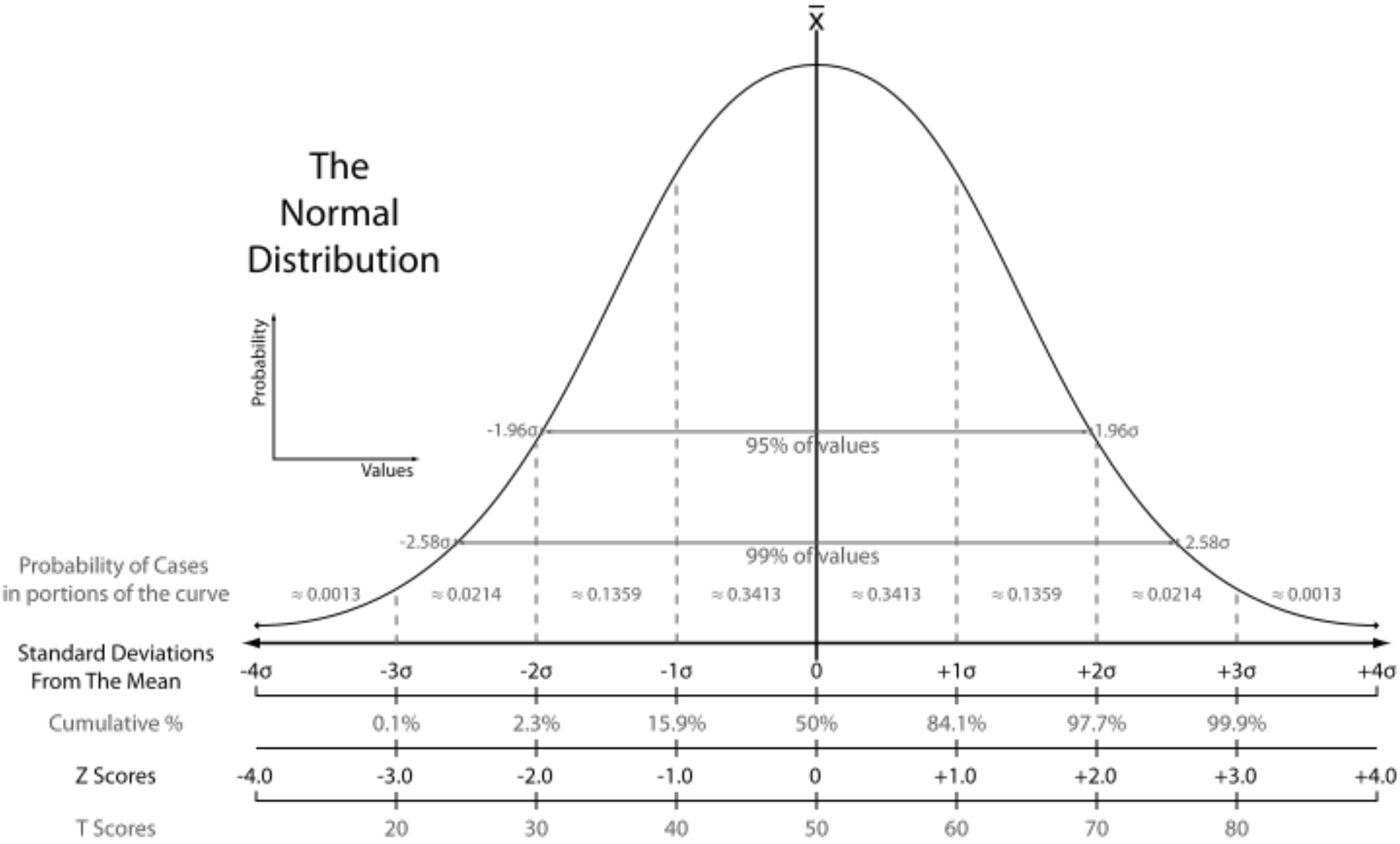
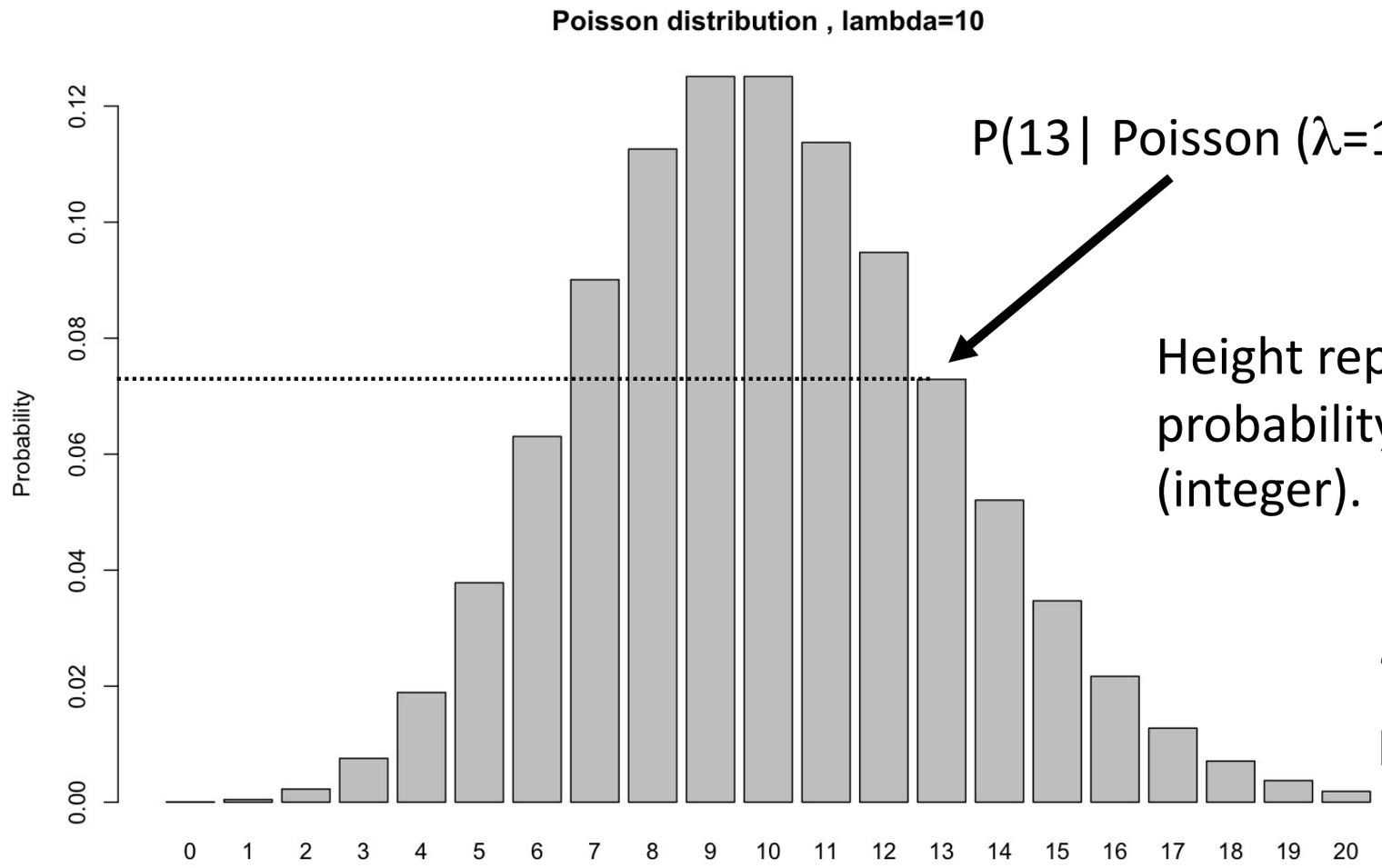
How to Statistics

Amanda Charbonneau

The Normal Distribution



Probability Mass function (For discrete distributions, like read counts)



P(integer) ≥ 0

P(non-integers) = 0.

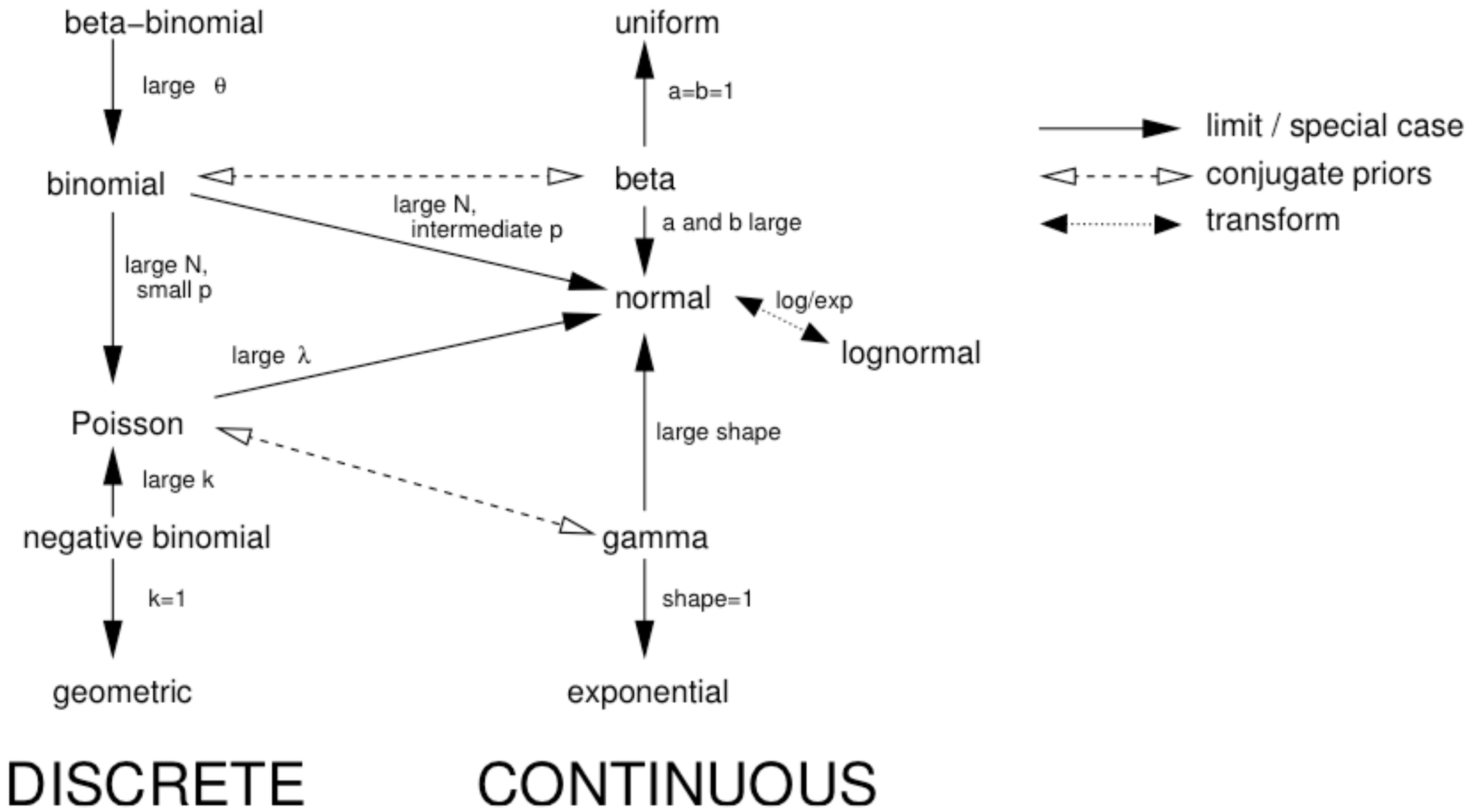


Figure 4.17 Relationships among probability distributions.

Bolker 2007 CH4 page 137

Negative binomial

$$\text{Negative Binomial Distribution} = \frac{\Gamma(k+x)}{\Gamma(k)x!}\left(\frac{k}{k+\mu}\right)^{k}\left(\frac{\mu}{k+\mu}\right)^{x}$$

Expected number of counts =μ
Over-dispersion parameter = k

For our purposes all we care about is that

$$\text{var}(x) = \mu + k\mu^2$$

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

–Ronald Fisher

I have an idea…

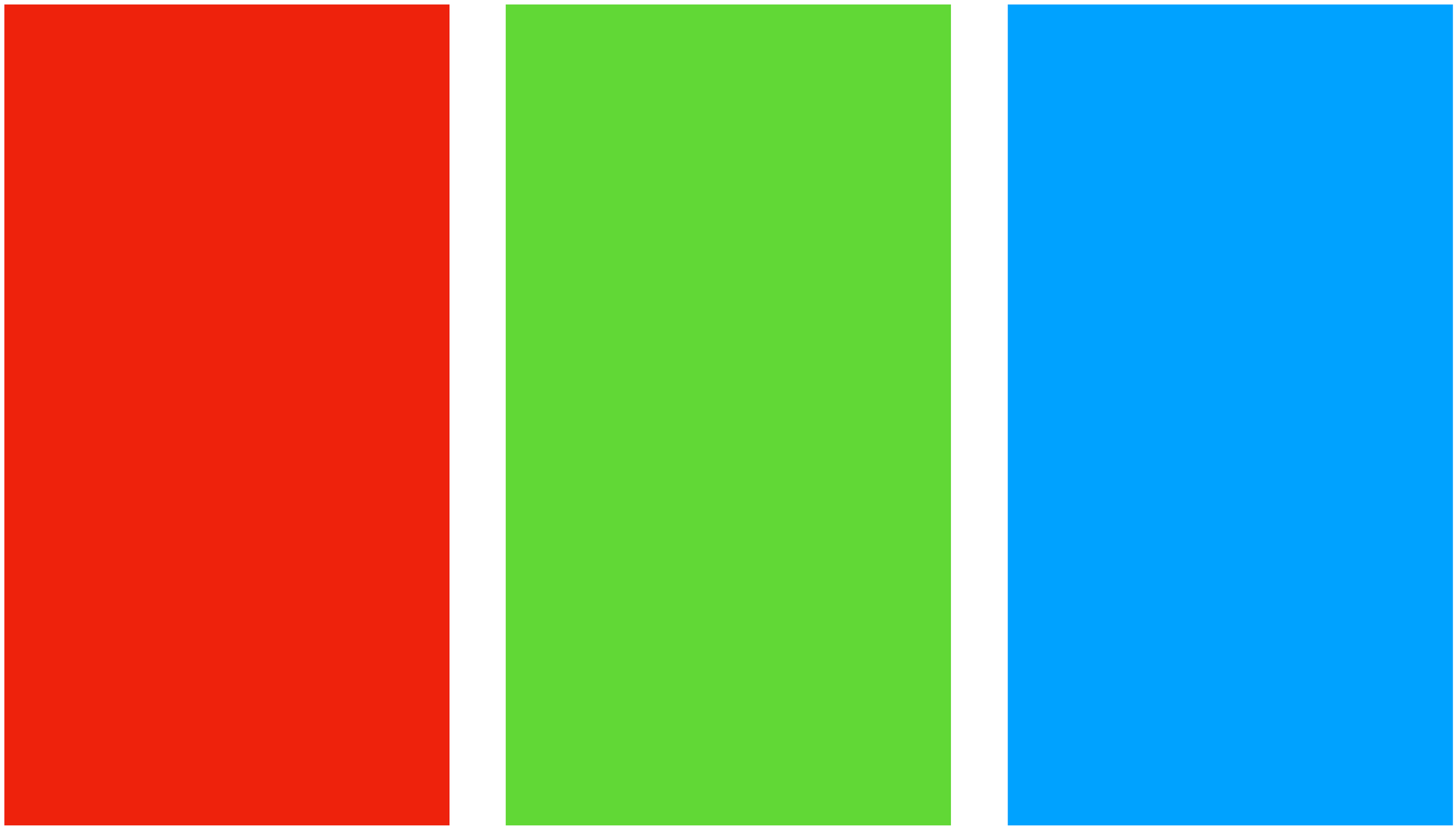
- 144 individuals
- 48 of each treatment
- Treatment lasts 1 week
- We have 3 incubators/ greenhouses/tanks/cages which each hold 48 individuals

Experimental Design

Place 1

Place 2

Place 3



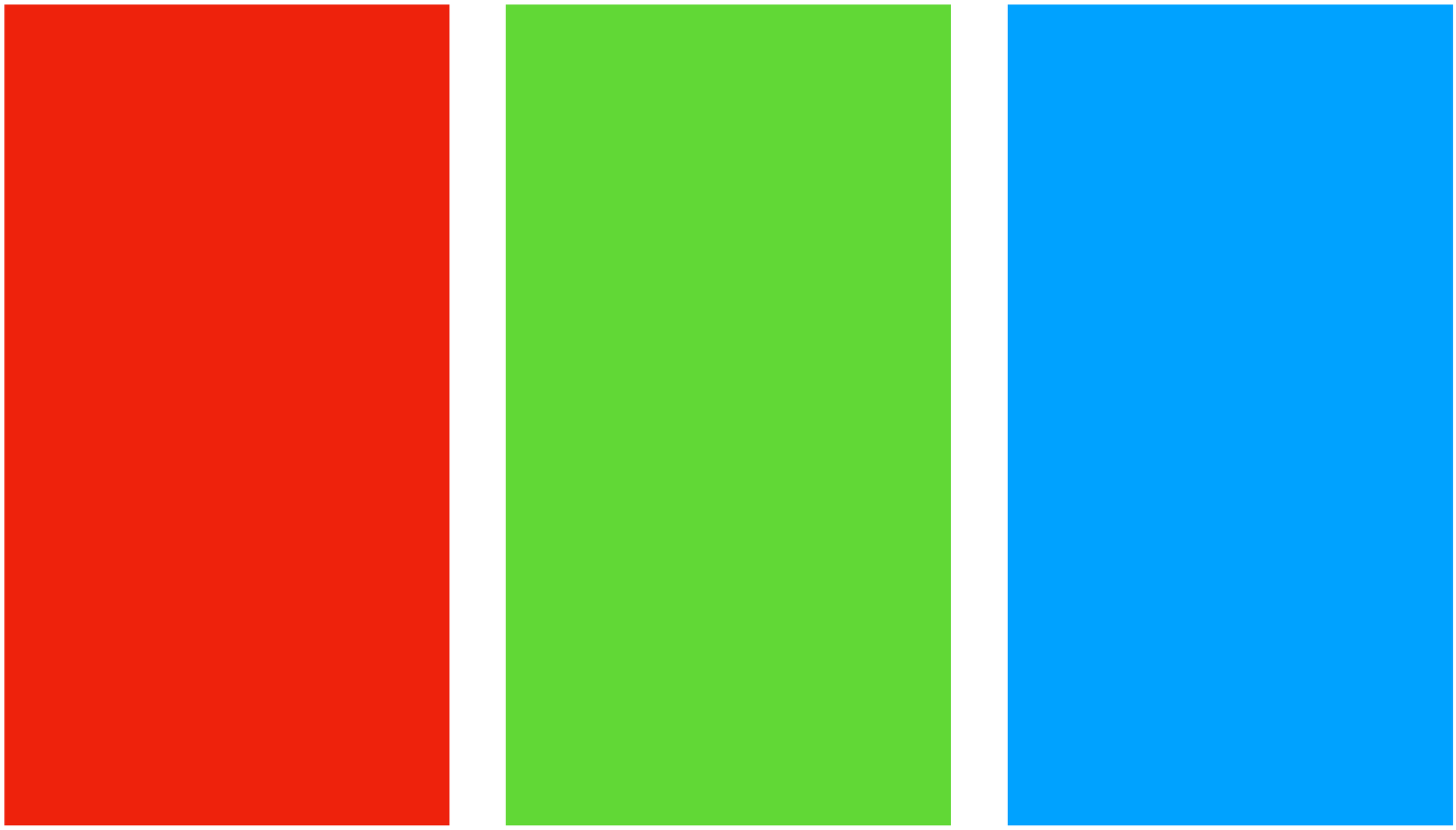
This is the plan. What do you think?

Experimental Design

Place 1

Place 2

Place 3



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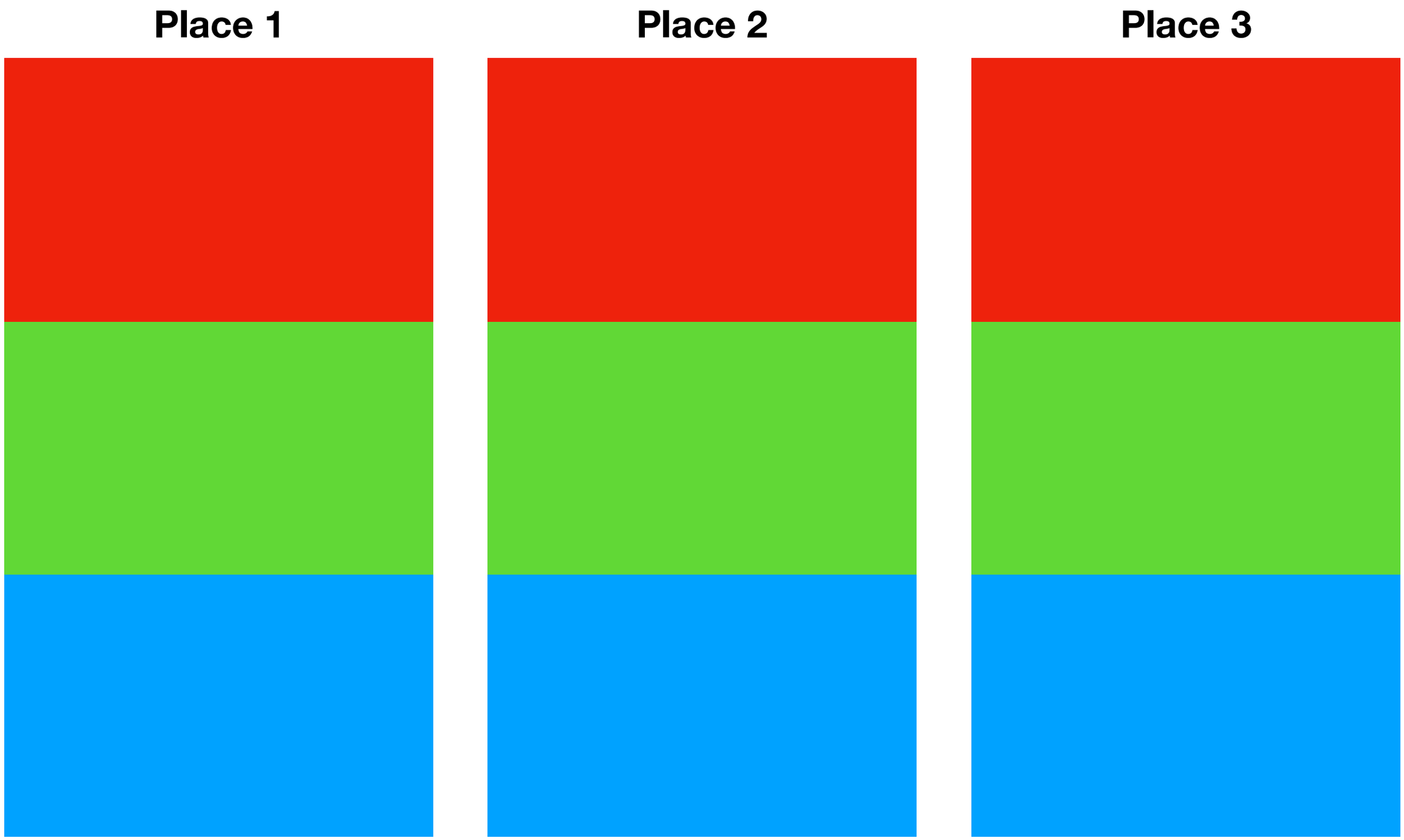
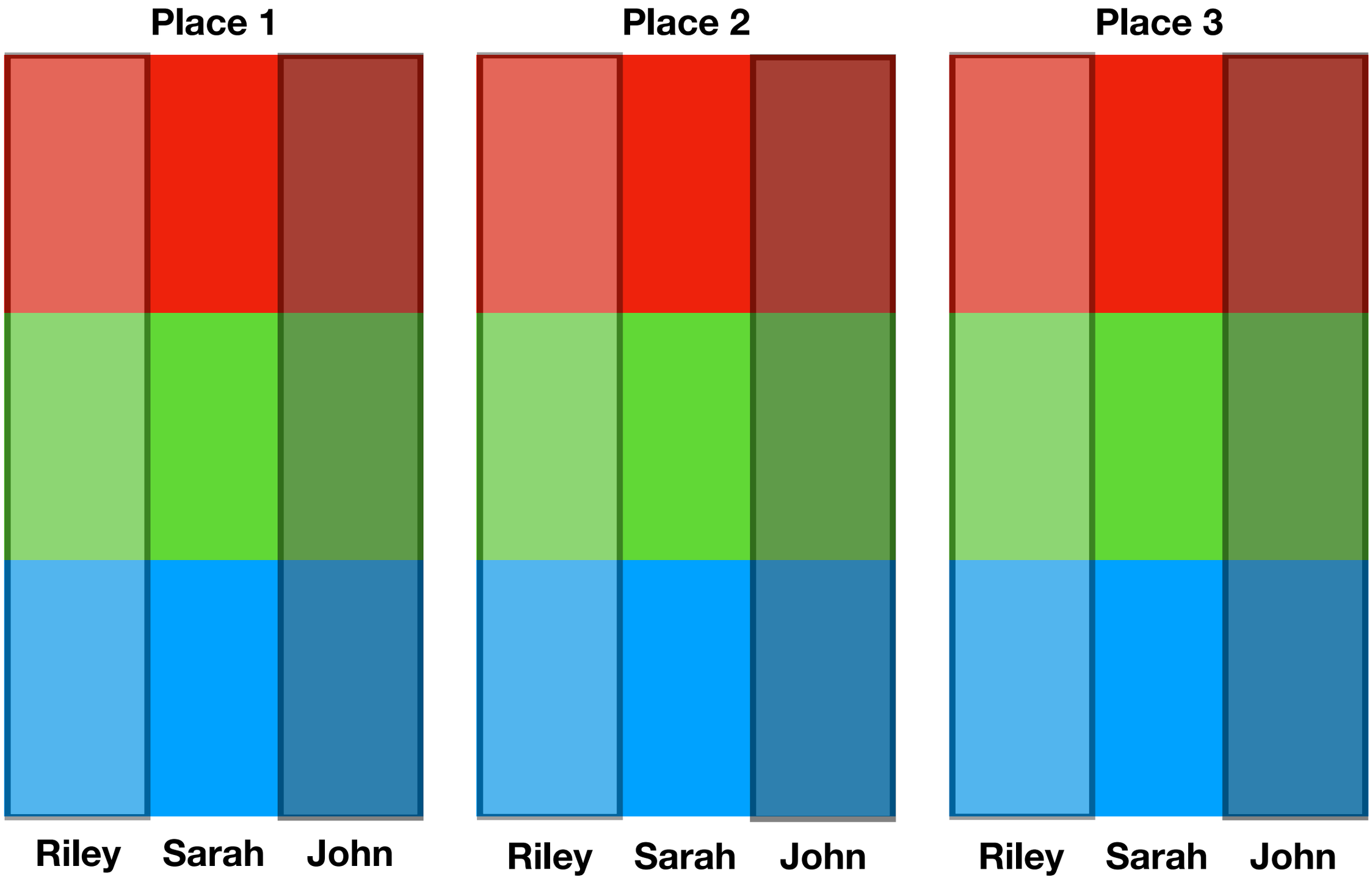


You have 3 undergrads. How should they split the data collection work?

Experimental Design

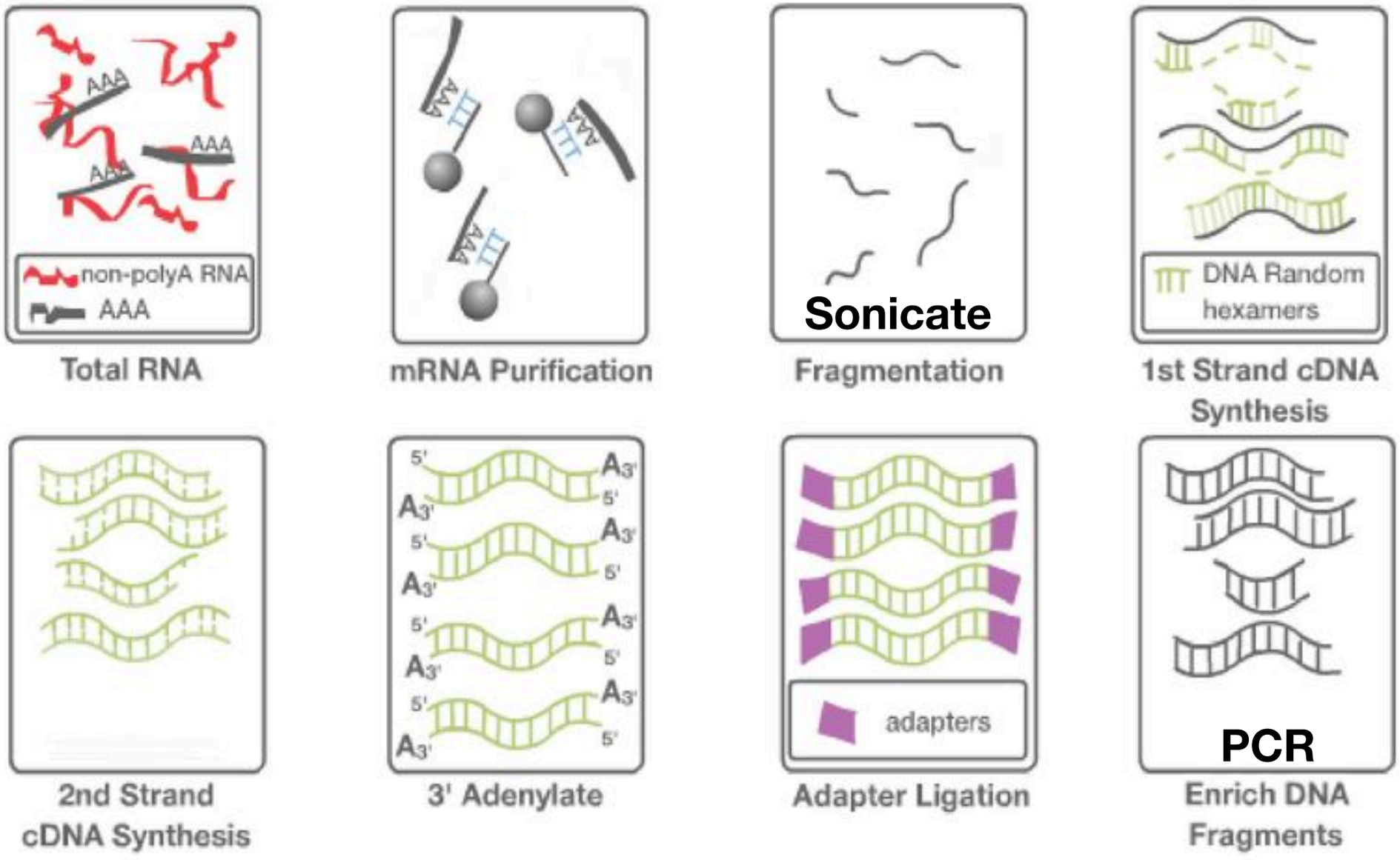


Experimental Design

Sample	Treatment	Place	Student	Measurement
1		1	Riley	92
2		1	Sarah	56
3		1	John	21
4		2	John	77
5		2	Riley	35
6		2	Sarah	26
7		3	Sarah	68
8		3	John	41
9		3	Riley	42

Let’s say you collect RNA for sequencing from all your organisms, what other variables should we record?

1. TruSeq® RNA Sample Prep Kit V2

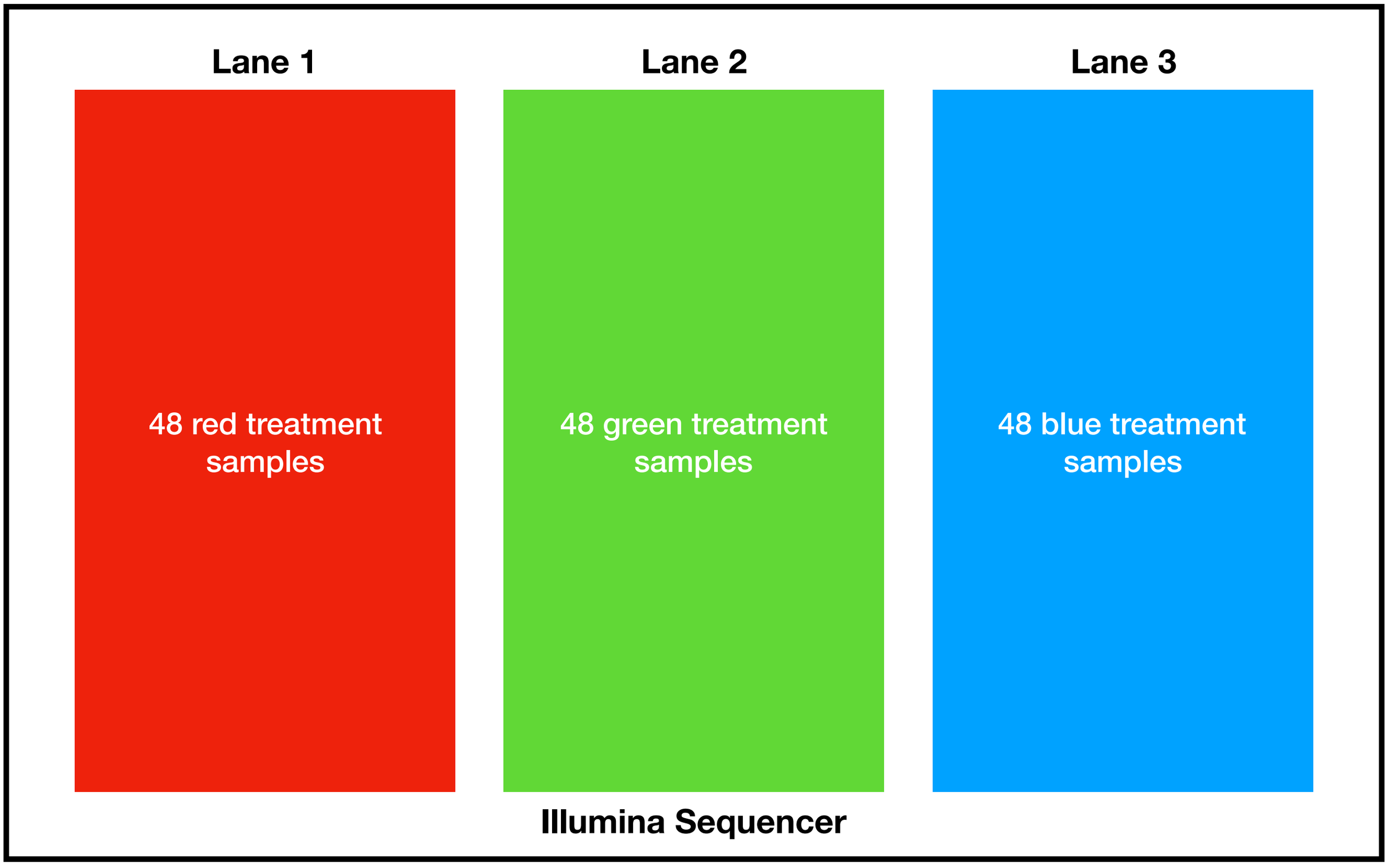


Most people can prep 8 to 16 samples at a time. A robot can do 96.

If this is going to be an RNAseq experiment, what variables should we try to account for?

- Time collected RNA
- Extraction method/batch
- Time in storage
- PCR differences
- When/where samples collected
- Kit number

Is this how you load your sequencer?



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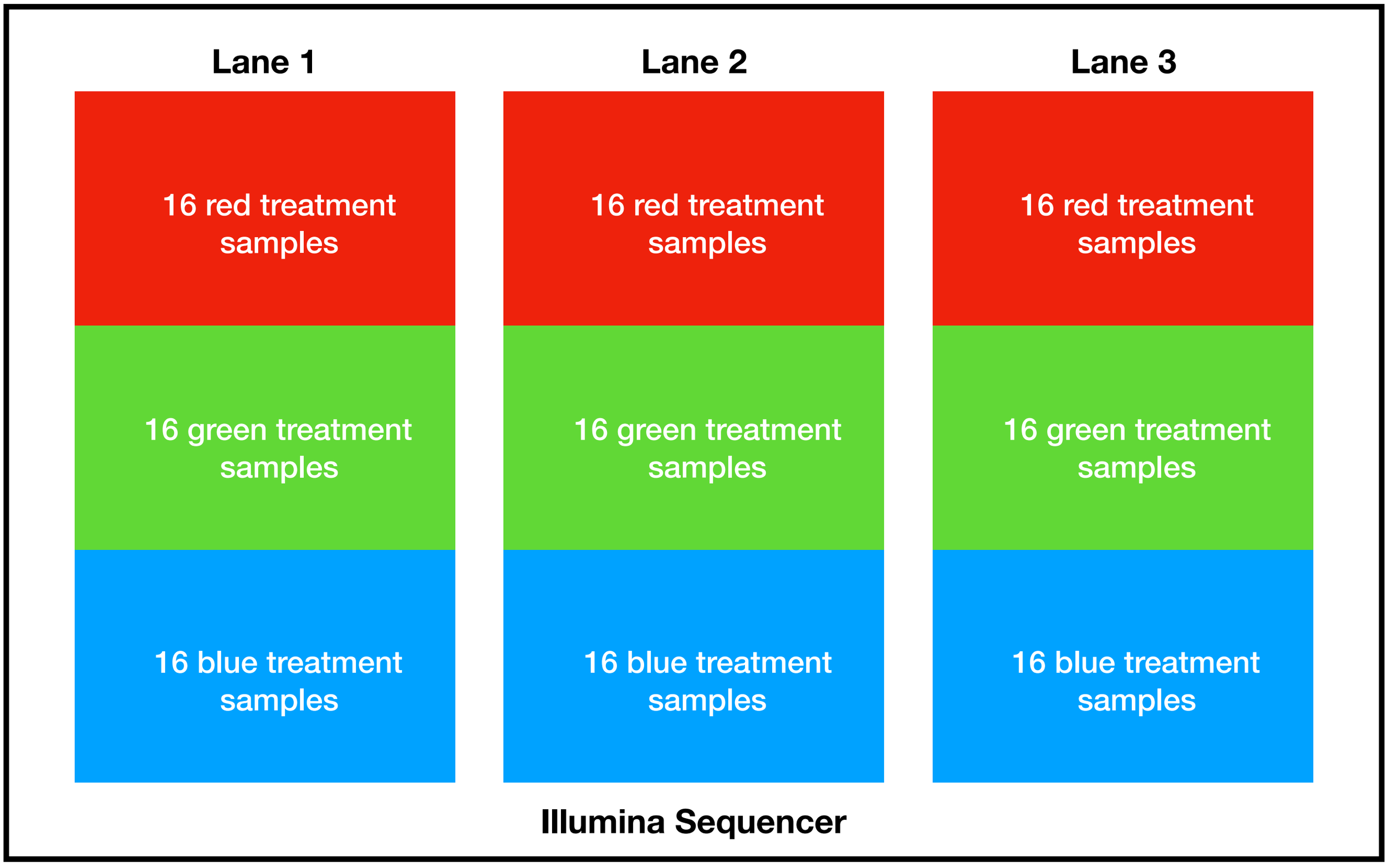
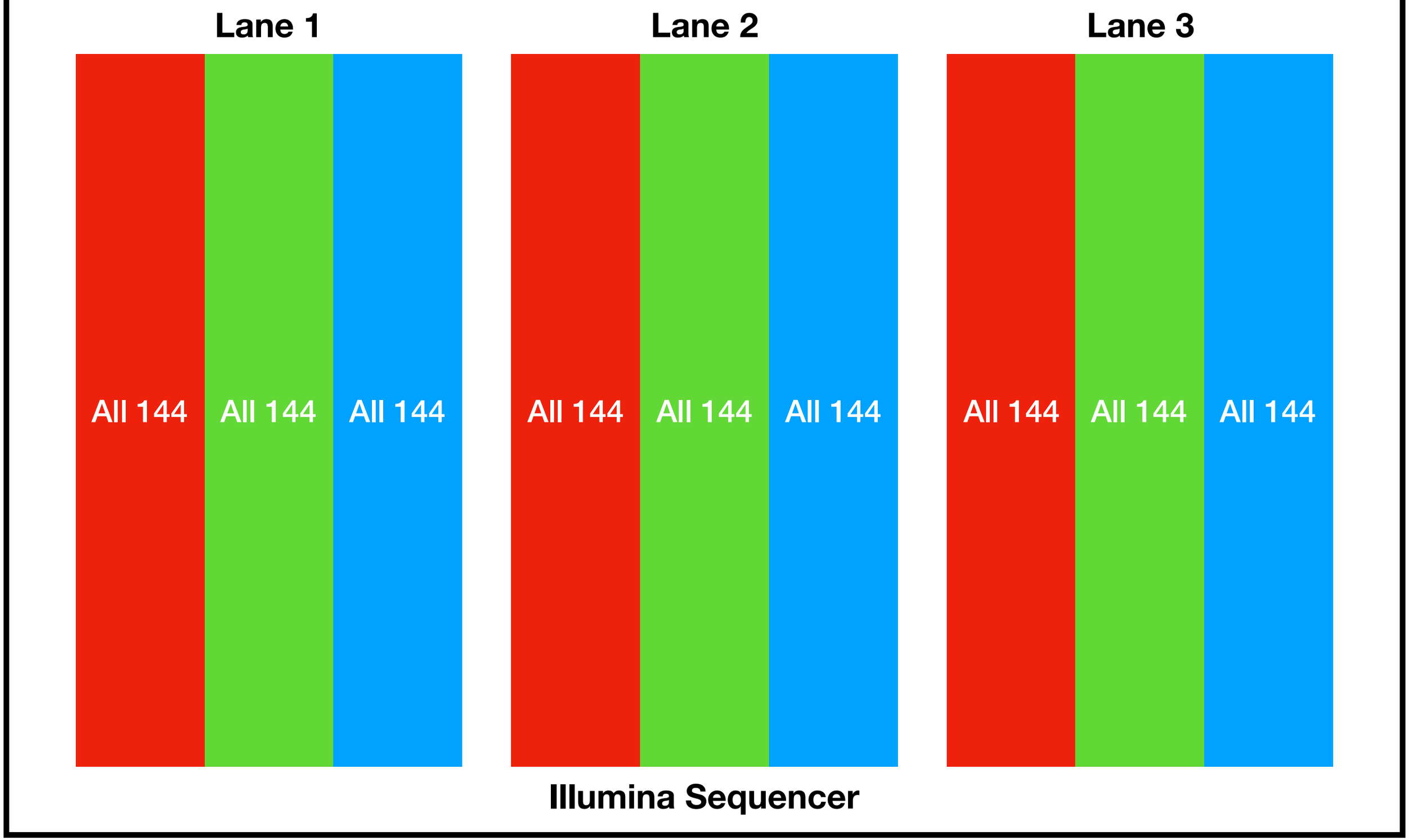


Experimental Design



If this is going to be an RNAseq experiment, what variables should we try to account for?

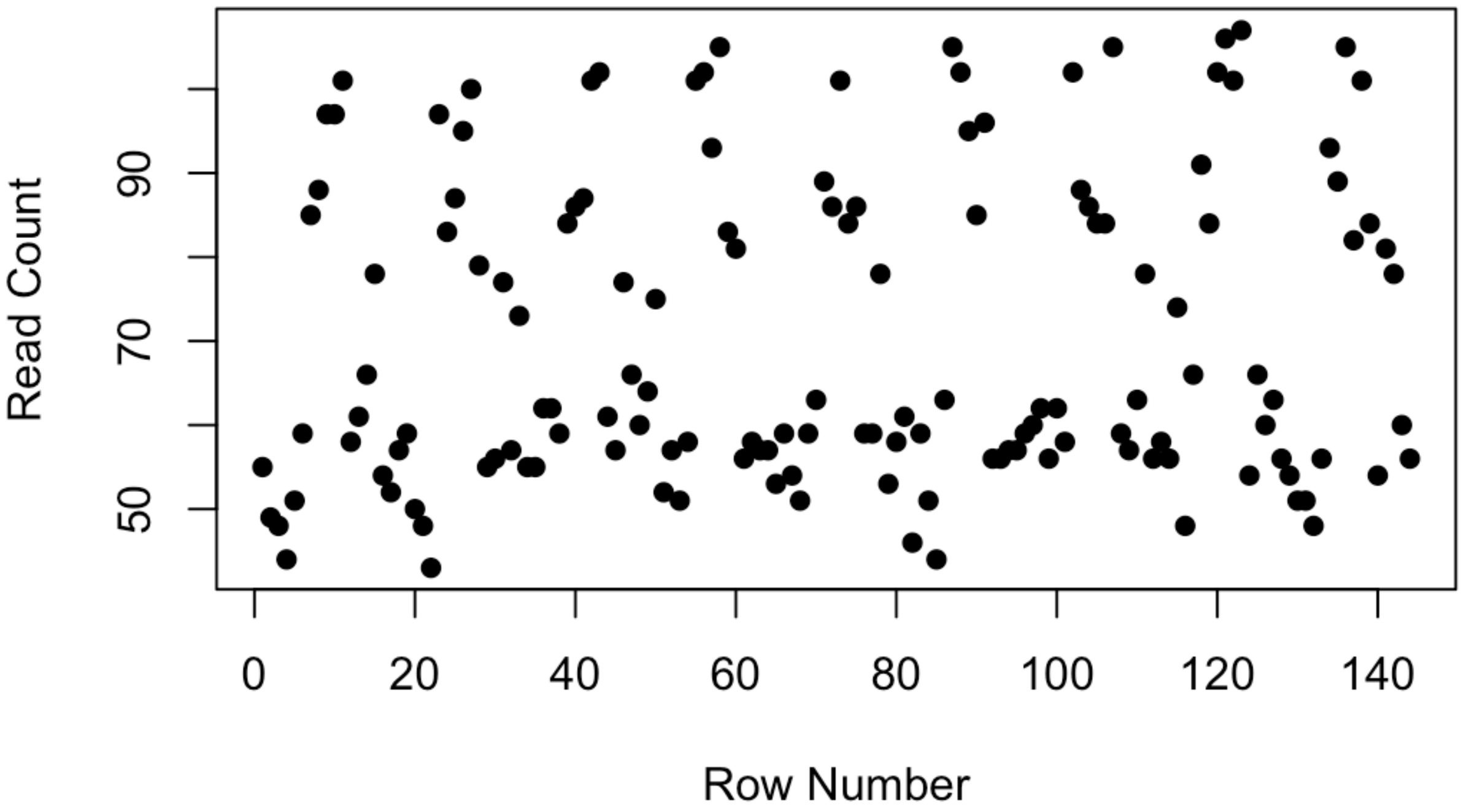
- Time collected RNA
- Extraction method/batch
- Time in storage
- PCR differences
- When/where samples collected
- Kit number
- Sequencing date
- Sequencer lane
- Index/custom barcodes
- Pooling
- Sequencing center
- Sequencer

RNAseq is an experiment, not an assay

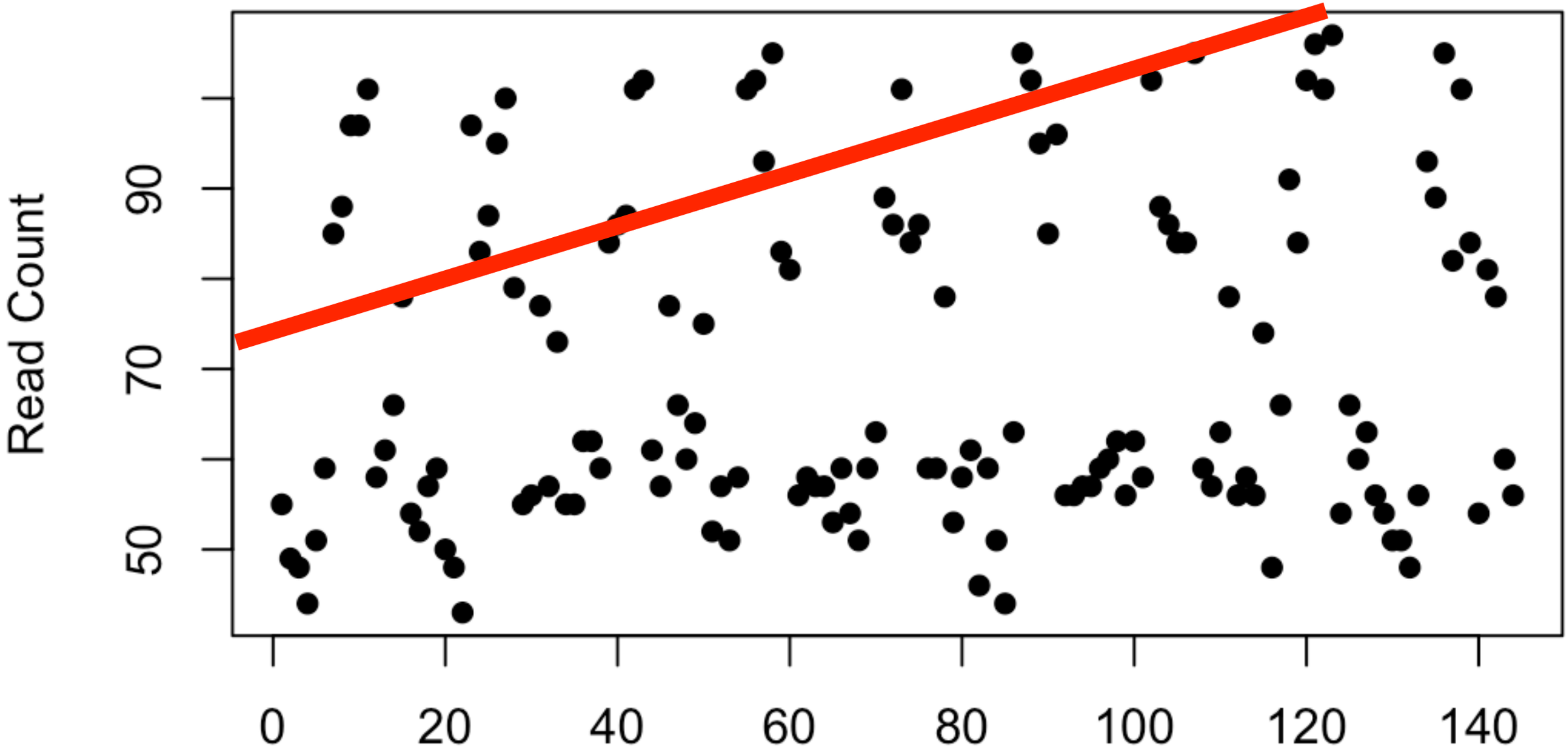
Experiment Matrix

Sample	Treatment	Place	Student	ReadCount	Lane	Library Prep Date
1		1	Riley	92	1	5/23/18
2		1	Sarah	56	2	6/30/18
3		1	John	21	1	5/23/18
4		2	John	77	2	6/30/18
5		2	Riley	35	1	5/23/18
6		2	Sarah	26	2	6/30/18
7		3	Sarah	68	1	5/23/18
8		3	John	41	2	6/30/18
9		3	Riley	42	1	5/23/18

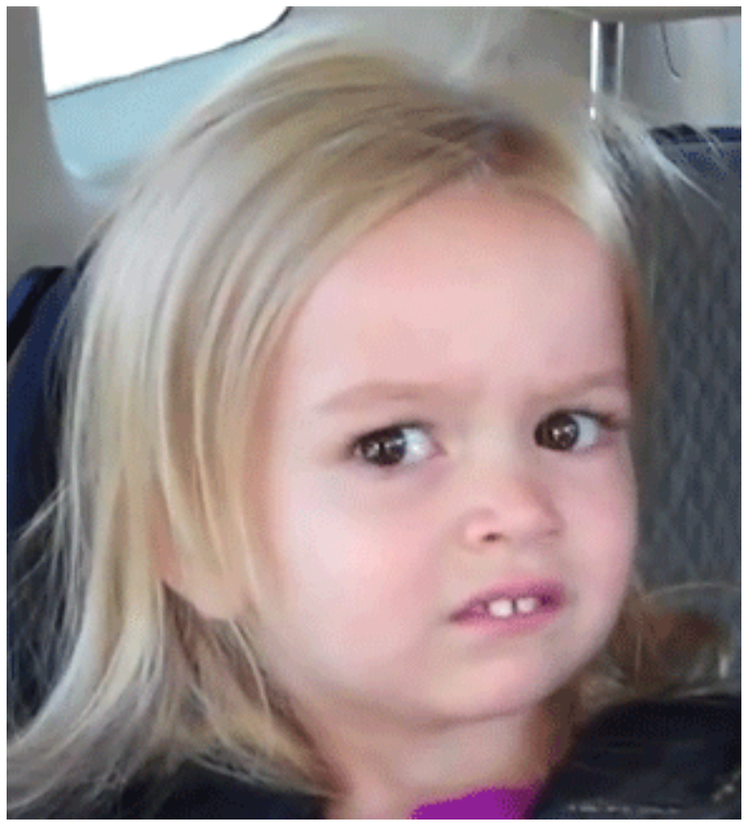
Our experimental data in a scatterplot



Our experimental data in a scatterplot, but now there's a line on it



How do you *feel* about that line?



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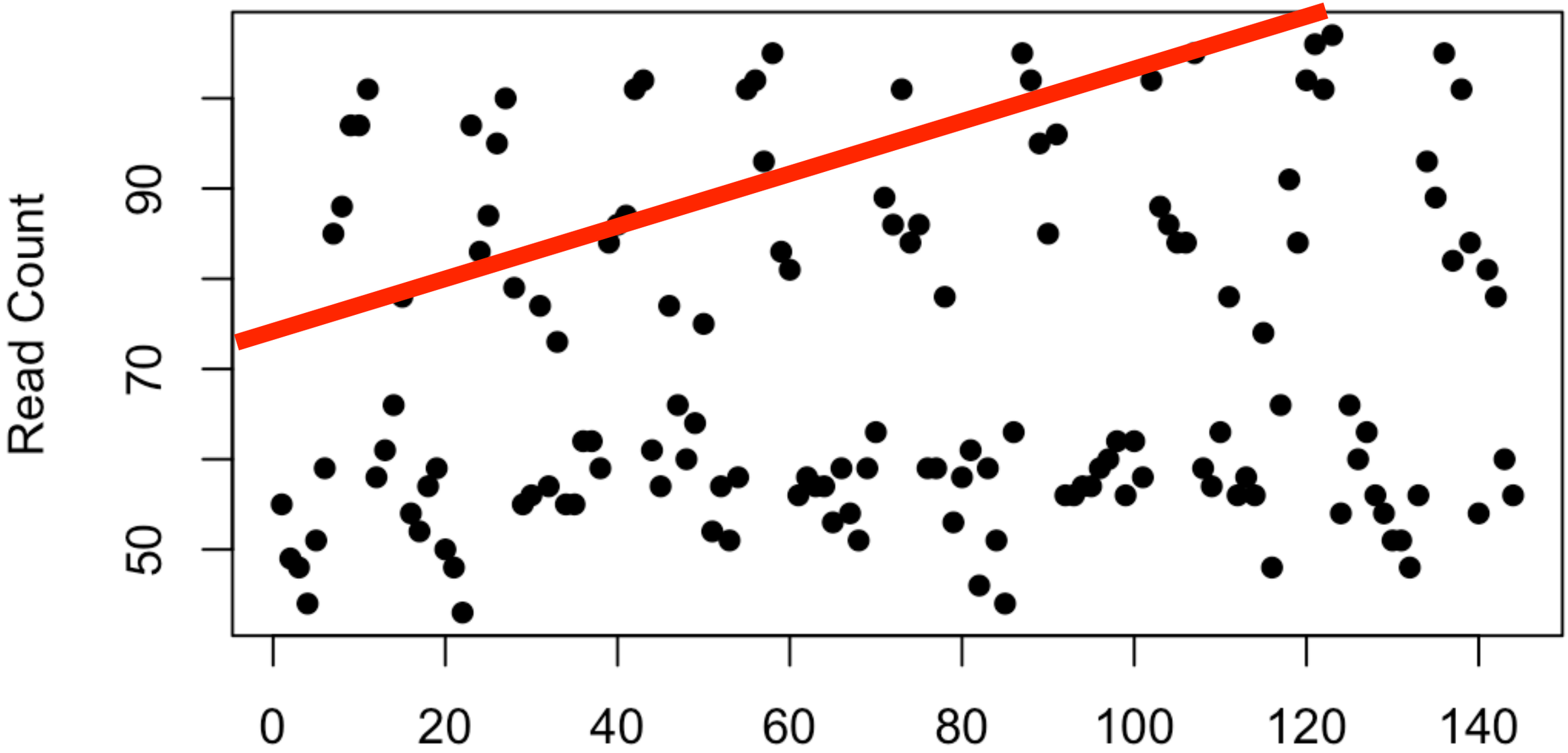
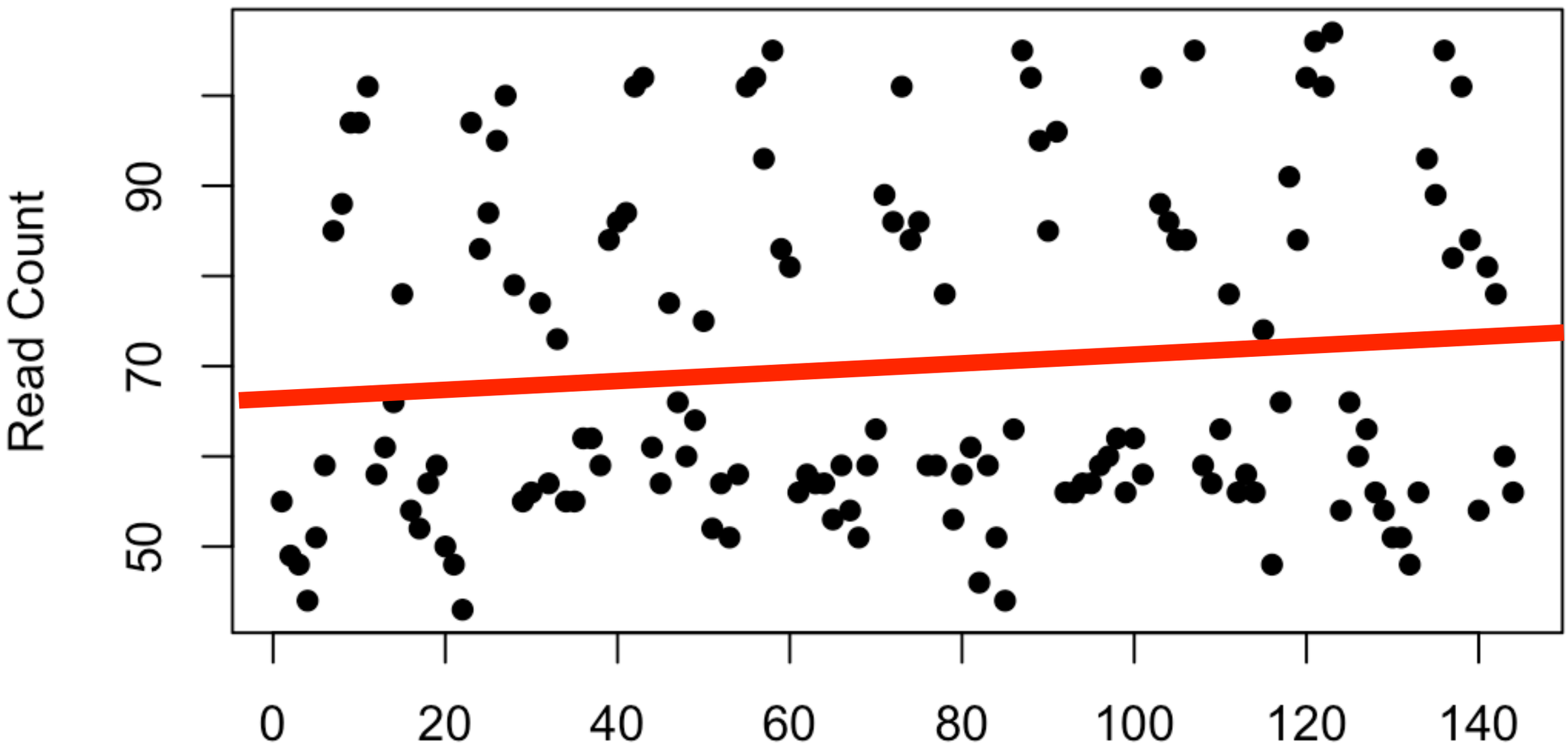


Why do you *feel* that way?

Our experimental data in a scatterplot, but now there's a line on it



Is this better? Why?

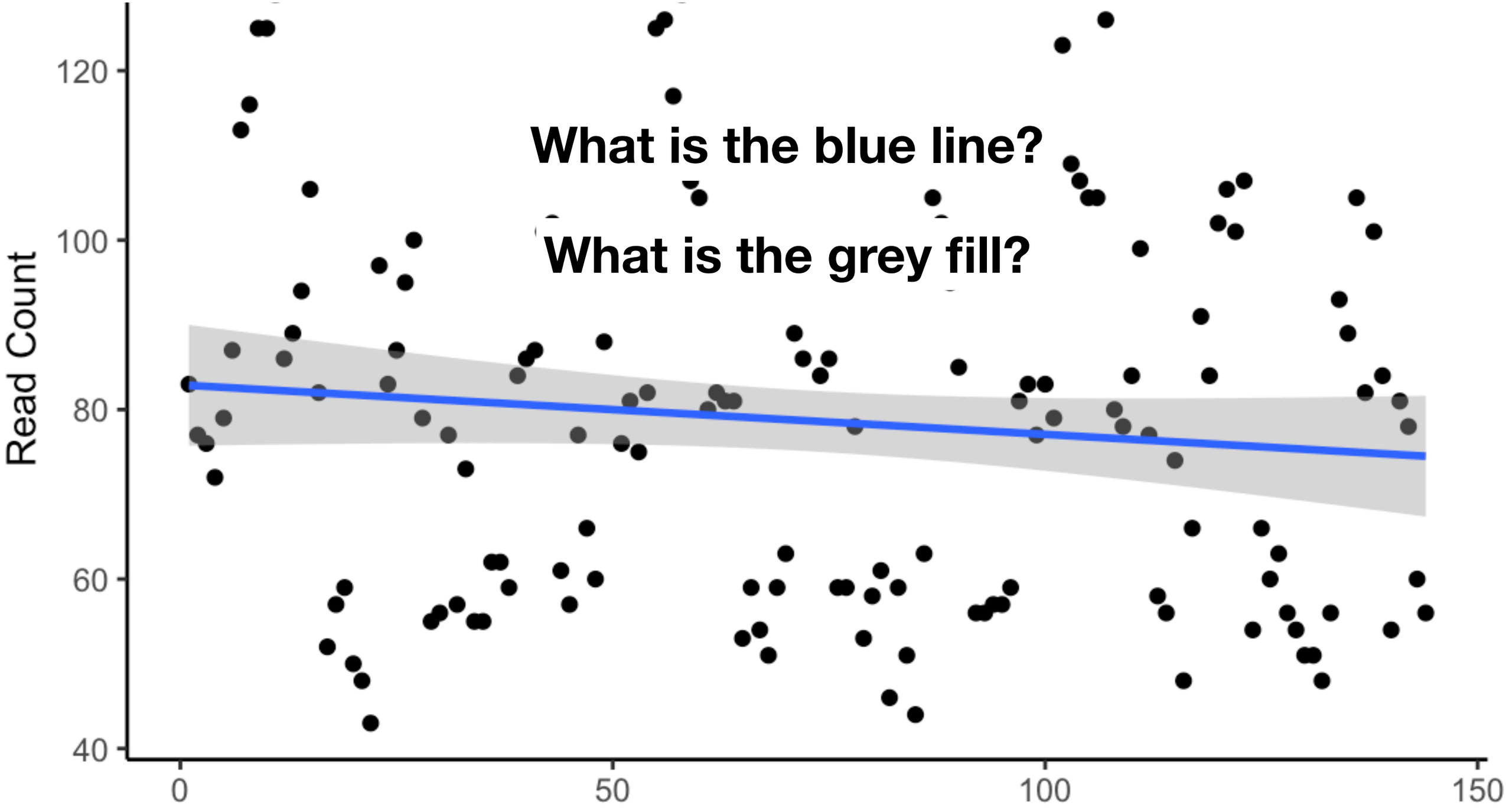
Proof we know math

- A ‘best fit’ line on a scatter plot is showing us the tendency of the dots…where their middle is, and what their slope is. It is the *average* of all those dots.

ReadCount ~ 1

What is the blue line?

What is the grey fill?



ReadCount ~ Treatment

Sample	Treatment	Place	Student	ReadCount	Lane	Library Prep Date
1		1	Riley	92	1	5/23/18
2		1	Sarah	56	2	6/30/18
3		1	John	21	1	5/23/18
4		2	John	77	2	6/30/18
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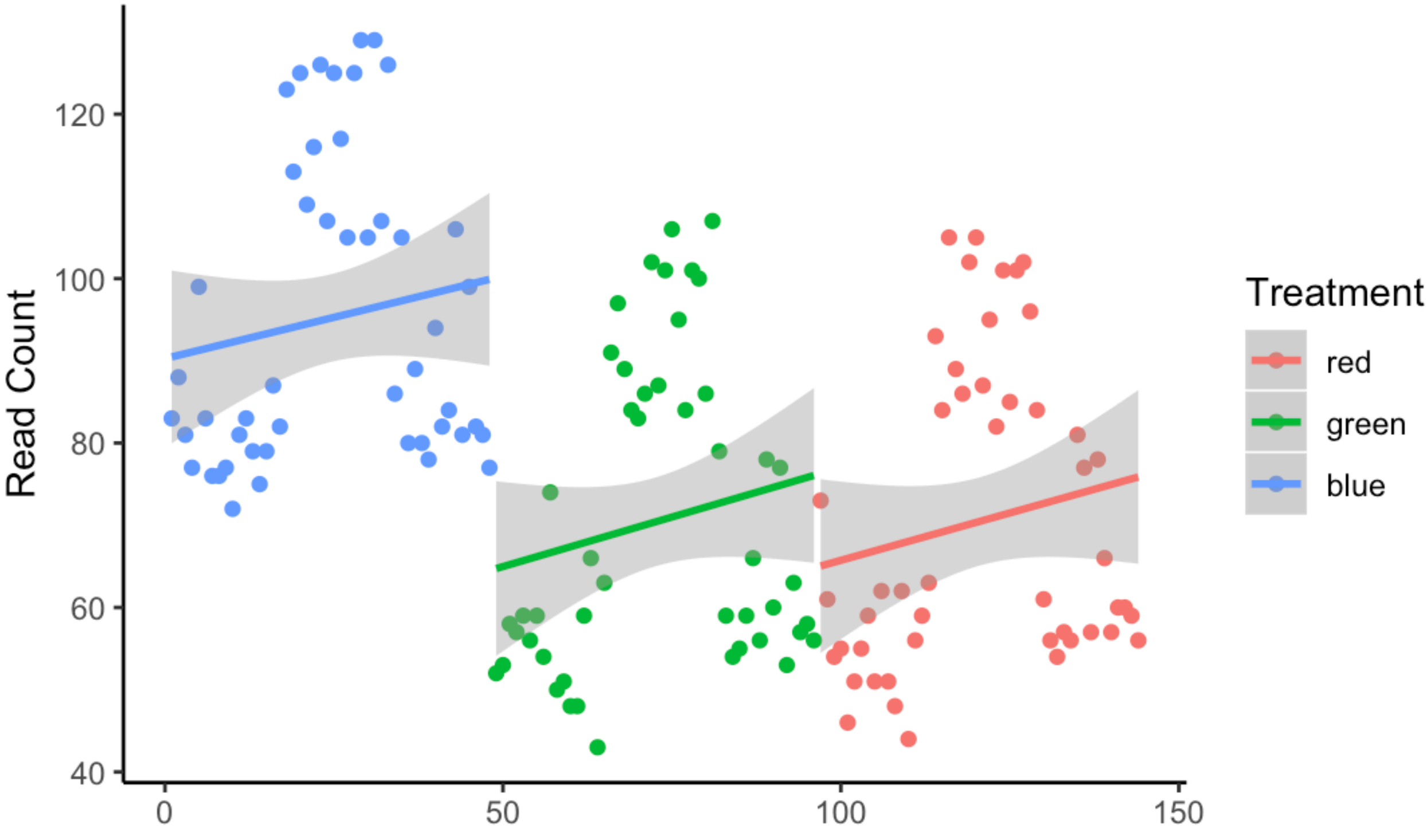
ReadCount ~ Treatment



Proof we know math

- A ‘best fit’ line on a scatter plot is showing us the tendency of the dots…where their middle is, and what their slope is. It is the *average* of all those dots.

- Mathematical modeling is really just estimating averages for the right data subsets.

ReadCount ~ Treatment + Student

Sample	Treatment	Place	Student	ReadCount	Lane	Library Prep Date
1		1	Riley	92	1	5/23/18
2		1	Sarah	56	2	6/30/18
3		1	John	21	1	5/23/18
4		2	John	77	2	6/30/18
5		2	Riley	35	1	5/23/18
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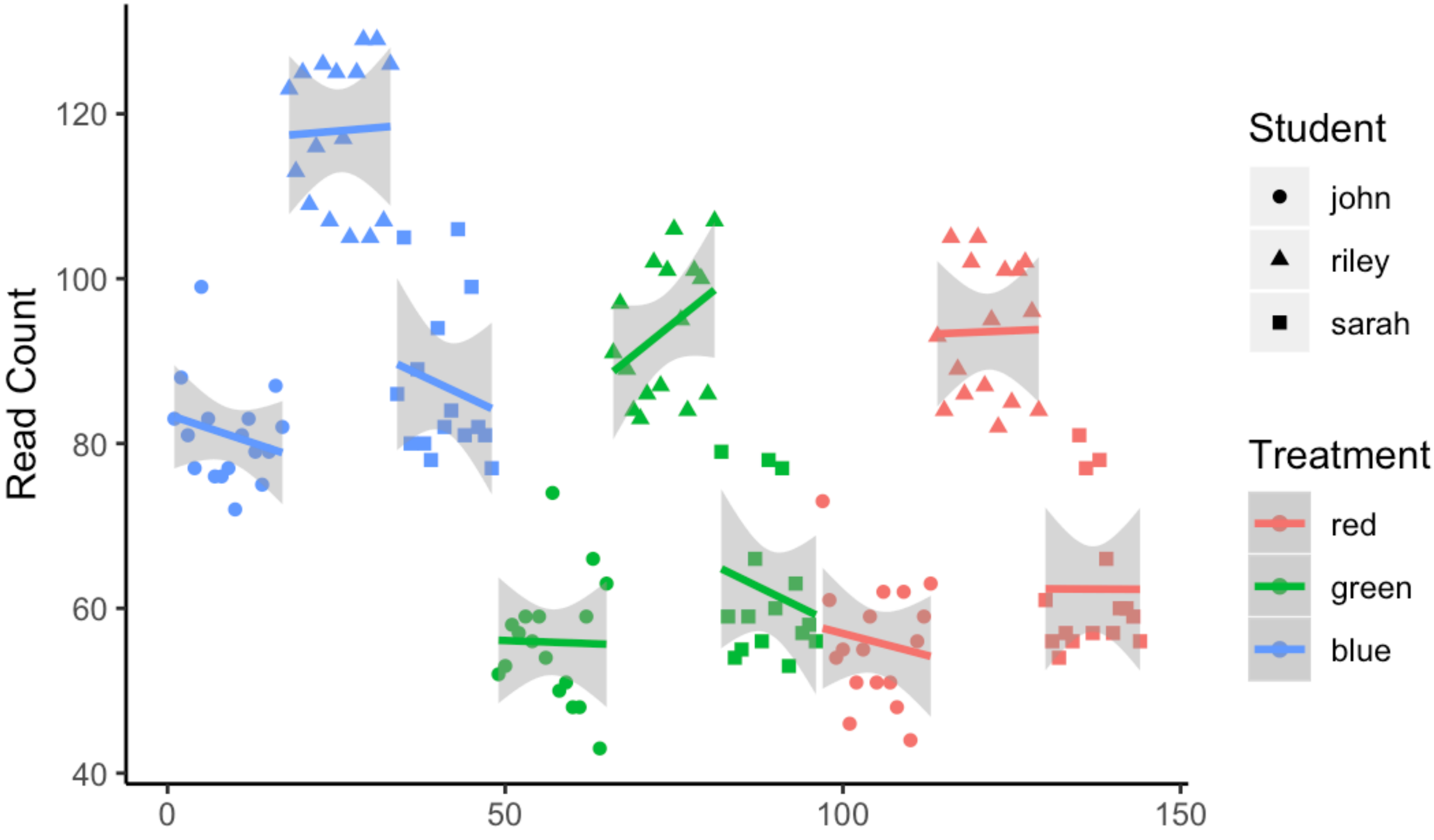
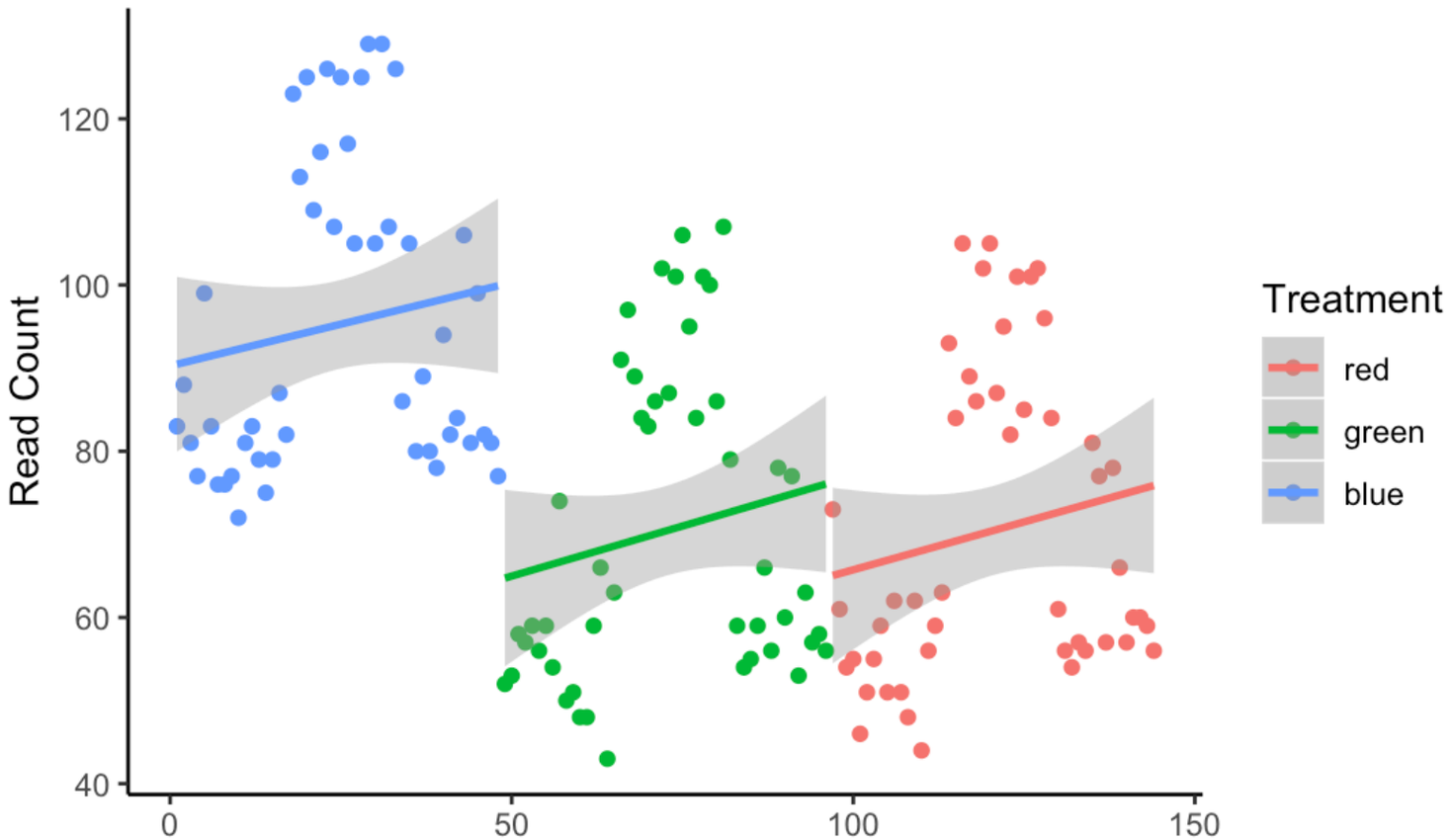
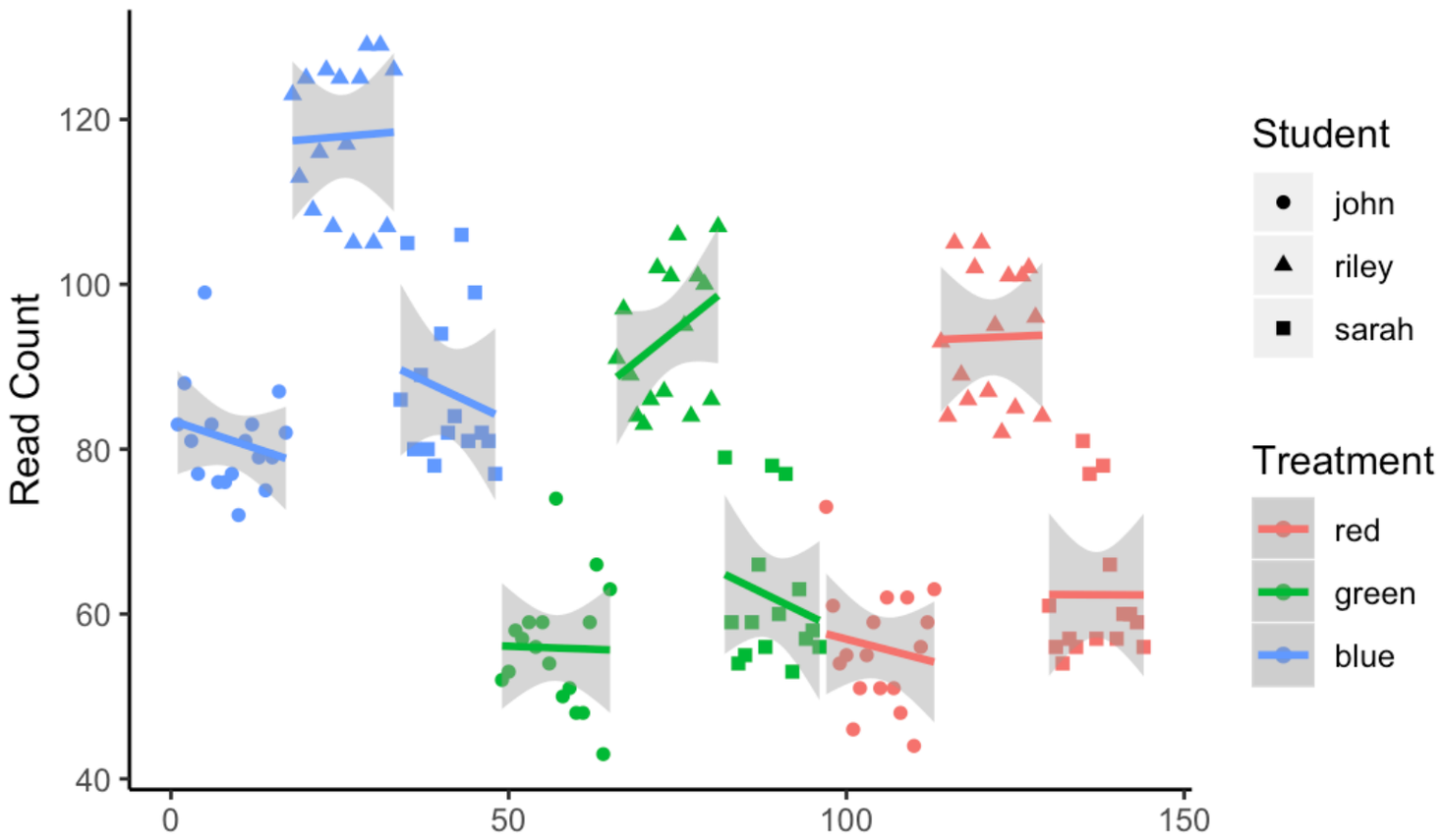
ReadCount ~ Treatment + Student



What is happening to my *data*?


Read Count
120
100
80
60
40
0
50
100
150
Treatment
red
green
blue


Read Count
120
100
80
60
40
0
50
100
150
Student
john
riley
sarah
Treatment
red
green
blue

ReadCount ~ Treatment + Place + Student

Sample	Treatment	Place	Student	ReadCount	Lane	Library Prep Date
1		1	Riley	92	1	5/23/18
2		1	Sarah	56	2	6/30/18
3		1	John	21	1	5/23/18
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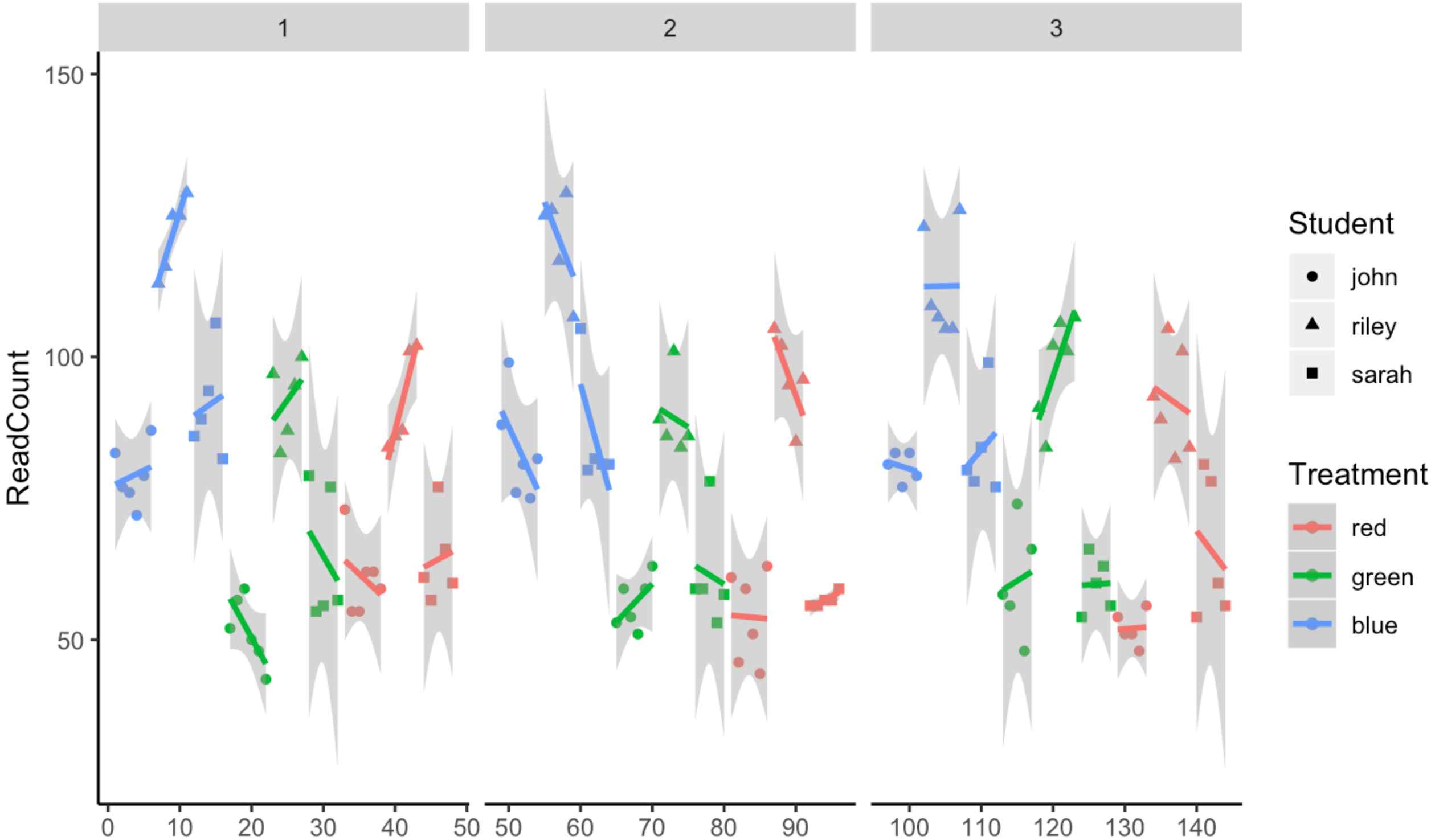
ReadCount ~ Treatment + Place + Student



What does this mean for your experimental design?

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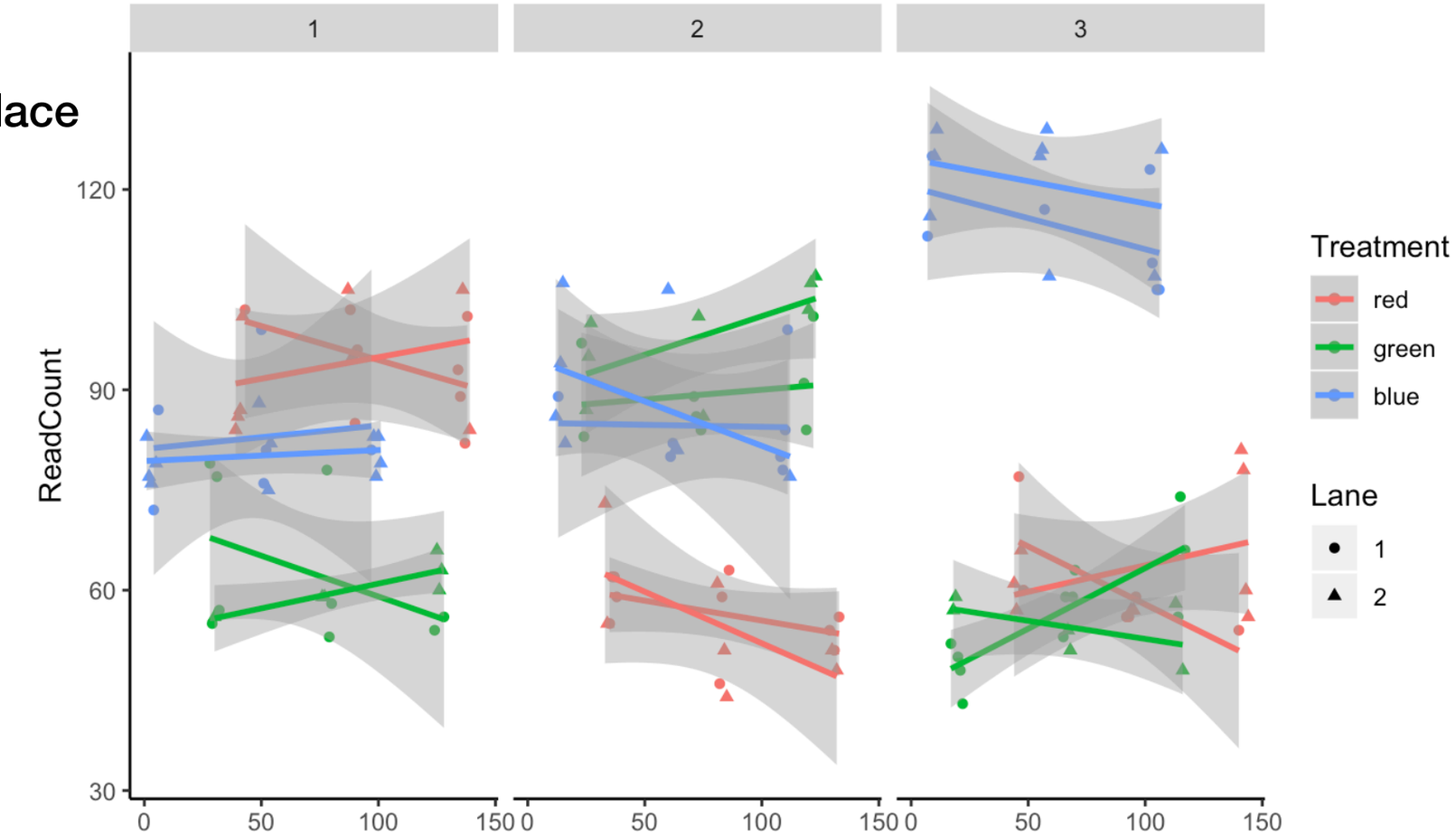
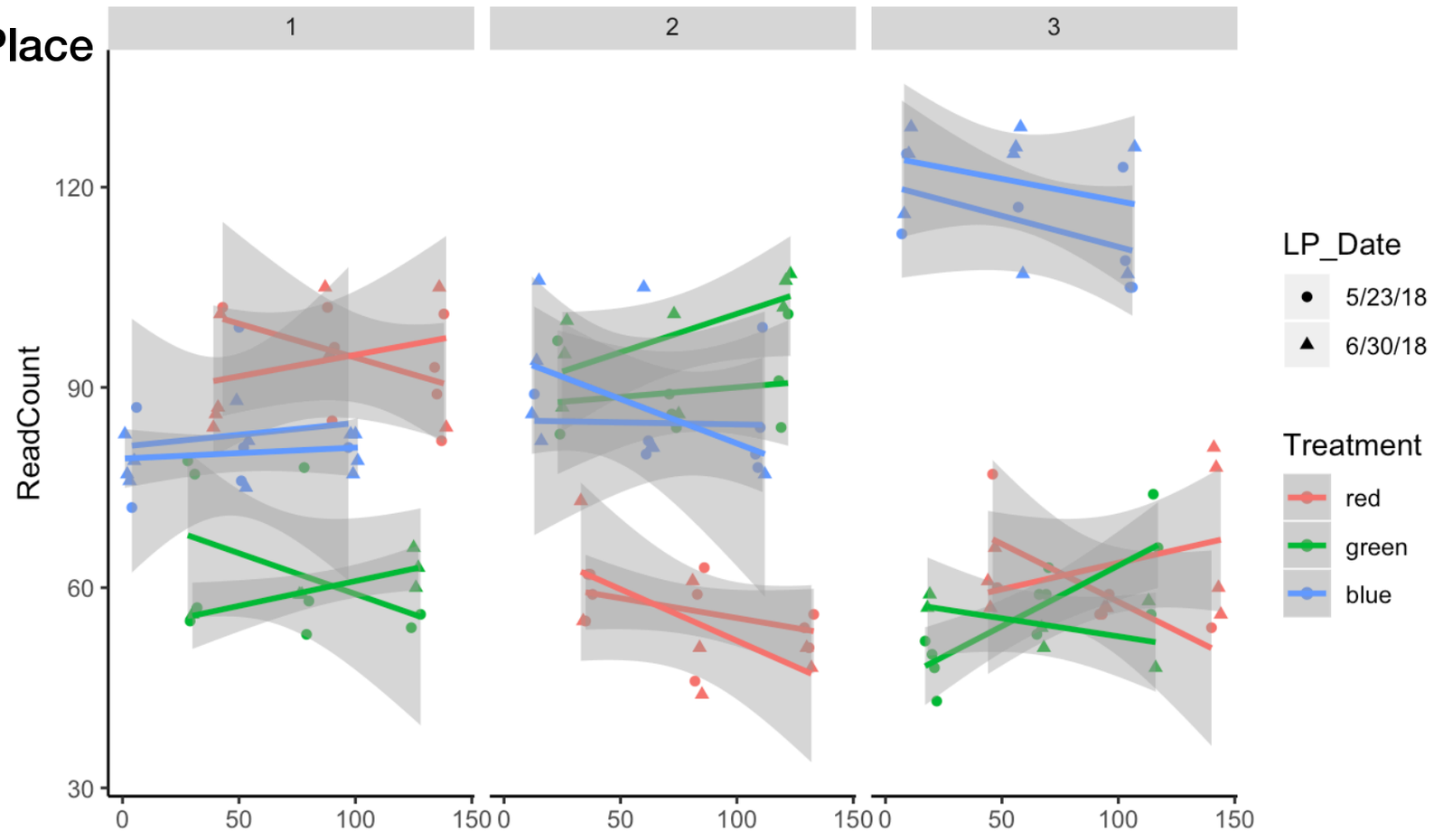
- Mathematical modeling is really just estimating averages for the right data subsets.

- A power analysis is figuring out how much data I need, to ask the question I care about, given the variables I’ll need to subset by. If I run out of points before I run out of subsets, I can’t answer my question.

ReadCount ~ Treatment + Lane + Place



ReadCount ~ Treatment + LP_Date + Place



Lane and LP_Date are perfectly confounded!

Sample	Treatment	Week	Student	ReadCount	Lane	Library Prep Date
1		1	Riley	92	1	5/23/18
2		1	Sarah	56	2	6/30/18
3		1	John	21	1	5/23/18
4		2	John	77	2	6/30/18
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- A power analysis is figuring out how much data I need, to ask the question I care about, given the variables I’ll need to subset by. If I run out of points before I run out of subsets, I can’t answer my question.

- My variables are confounded if they’re both averaging over the same points.

How many variables is ‘enough’?

Sample	Treatment	Week	Student	ReadCount	Lane	Library Prep Date
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8		3	John	41	2	6/30/18
9		3	Riley	42	1	5/23/18

Which model should we use?