Tyler Gandee

Demystifying the black box of sequencing: incorporating MinION sequencing of chloroplast genomes in the classroom
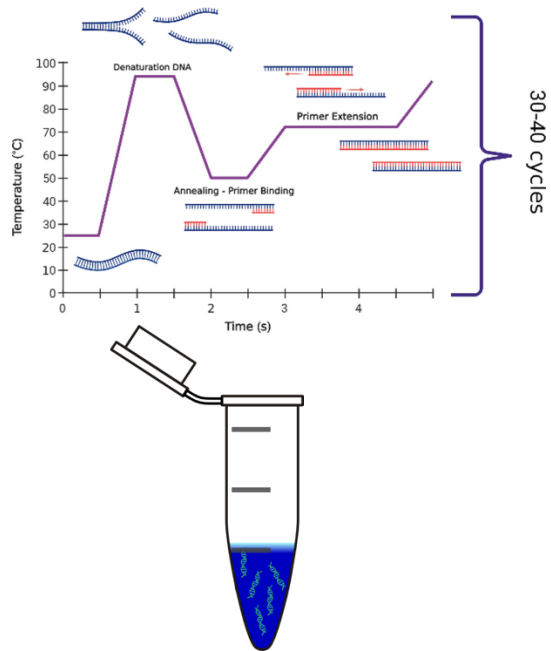
Karen Barnard-Kubow

James Madison University

September 2024

Traditional classroom genomic assembly pipeline

Sample collection, DNA extraction and amplification
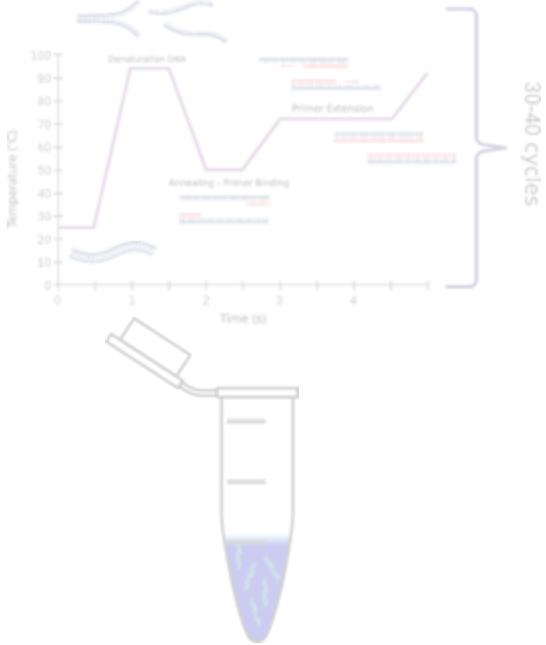

Send amplified DNA to a company to be sequenced

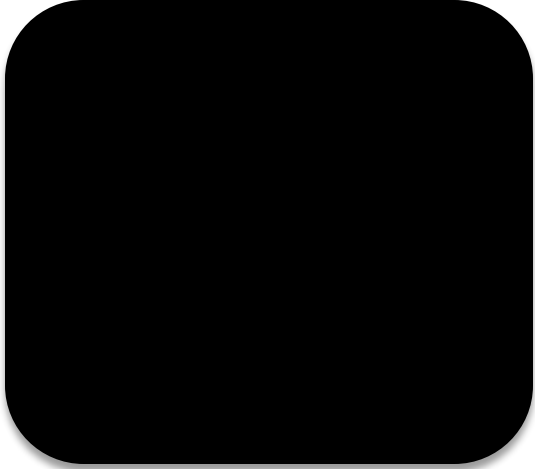Perform bioinformatics analysis on sequenced DNA

Current Problems with using Sequencing in the Classroom

Sample collection, DNA extraction and amplification

Sequencing is taught but
Send amplified DNA to a
rarely performed
company to be sequenced

Perform bioinformatics analysis on sequenced DNA

Possible way to combat the black box

Oxford Nanopore's MinION

Portable

Cost effective
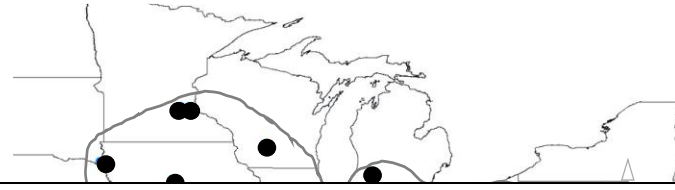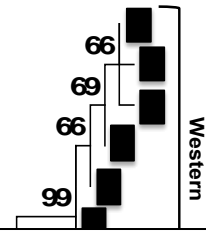
Easy libary prep

Quick set up for sequencing

Goals

- Develop a lab module that conveys core ideas about speciation and evolution using active learning and modern genomics.

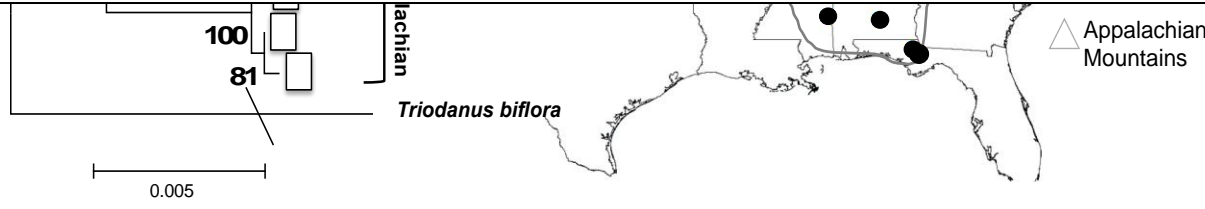- Directly test the impact of sequencer presence in the classroom.

Campanula americana
(American Bellflower)

Is there a relationship between plastid genome structural variation and strength of cytonuclear incompatibility?

66
69
66
99
Western

100
81

Triodanus biflora

0.005

Appalachian
Mountains

Western x
Eastern

Eastern x
Appalachian

Western x
Appalachian
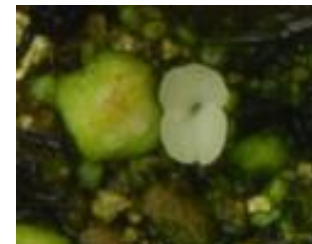
Module Design
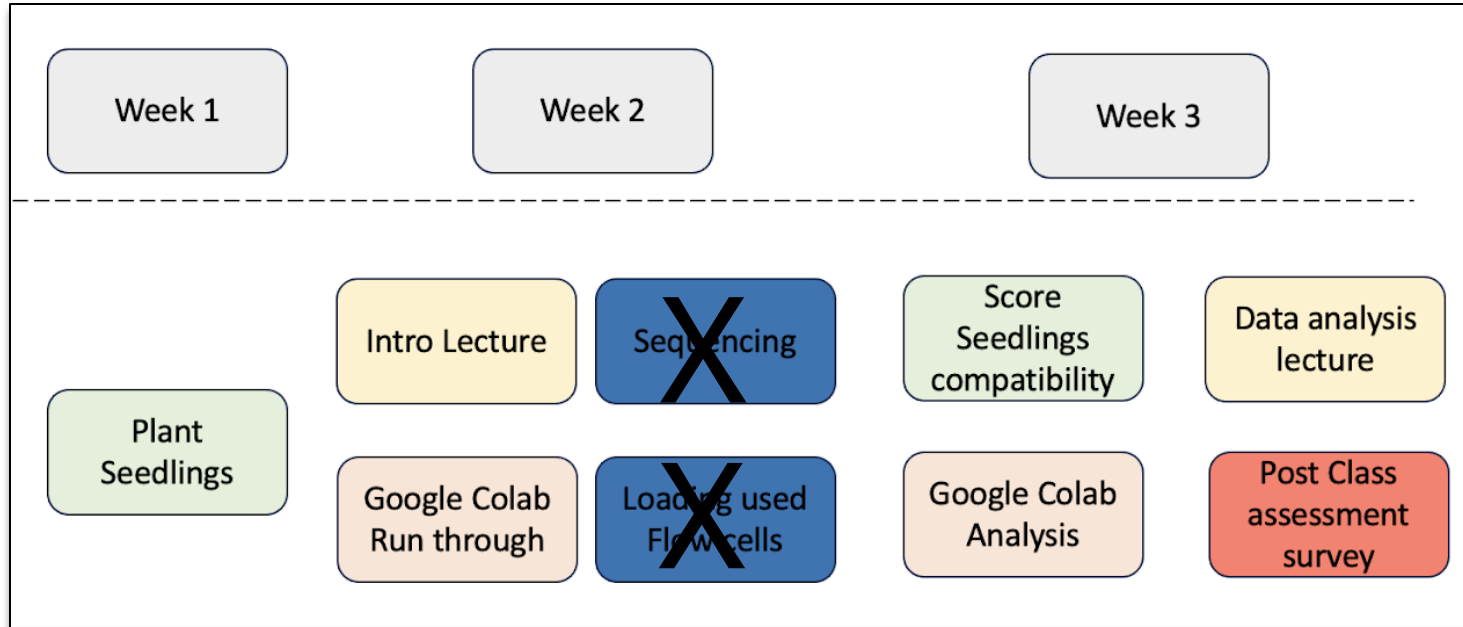
- Fall 2023 and Spring 2024 – 10 lab sections each
- Five sections with the sequencer
- Five sections without

3-week Module

Performed in the genetic lab courses at JMU

Course was piloted during Spring 2023

Fully implemented during Fall 2023 and Spring 2024

All Students

Planted F1 hybrid seed on soil plates
to assess incompatibility

Introductory Lecture

Students that had the Minion Present

Command Line Scripting/ Google colab

Examine the quality of your Minion reads using NanoPlot.

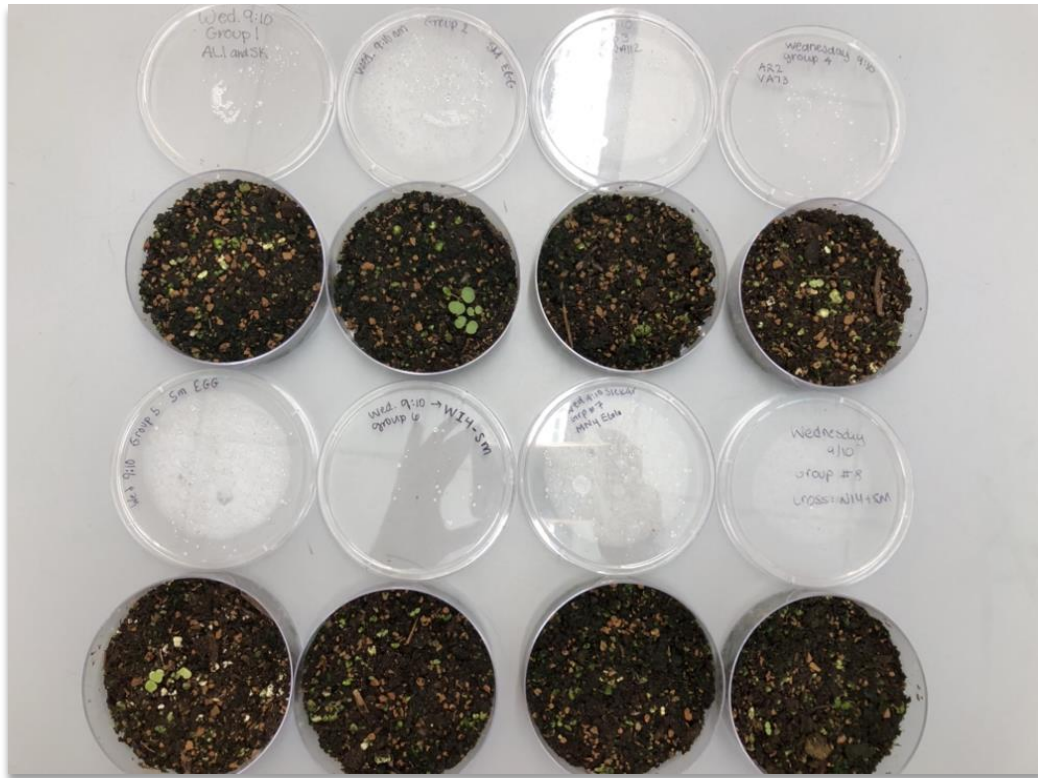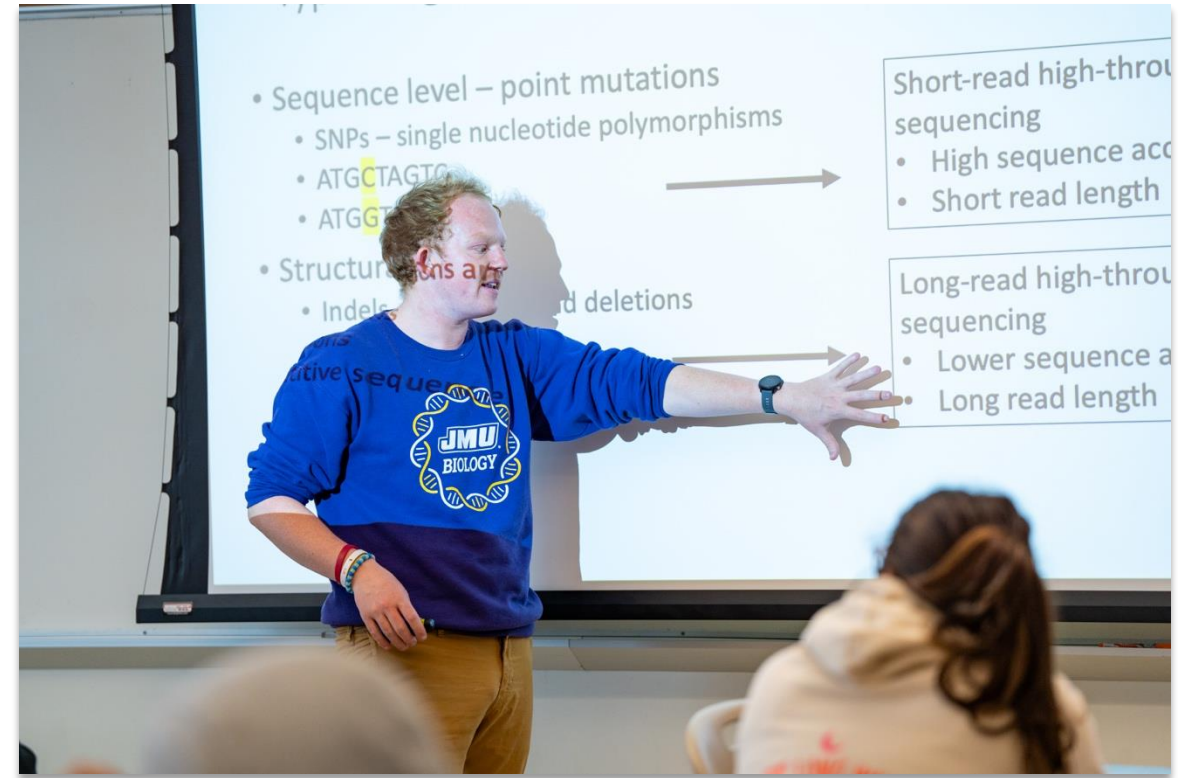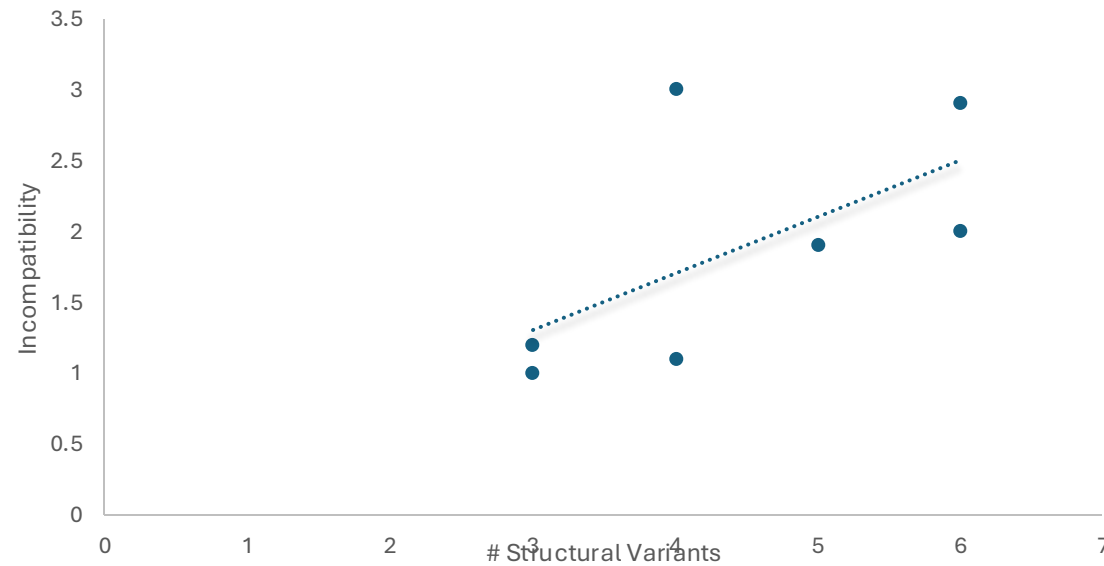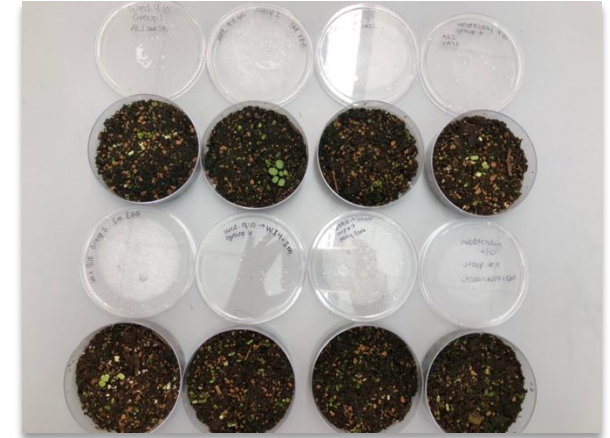First, we will install NanoPlot using the code below. Go ahead and run the code by hovering your mouse over the grey code block and clicking on the triangle play button. You will see output appear below as the program is installed.

```
[ ]   !pip install NanoPlot
```



```
[1]   !pip install NanoPlot

      Collecting NanoPlot
        Downloading NanoPlot-1.42.0.tar.
        Preparing metadata (setup.py) ..
      Collecting biopython (from NanoPlo
        Downloading biopython-1.83-cp310-

      Collecting pysam>0.10.0.0 (from Na
        Downloading pysam-0.22.0-cp310-c
      Requirement already satisfied: pan                      anoPl
      Requirement already satisfied: num                      anoPl
      Requirement already satisfied: scipy in /usr/local/lib/python3.10/dist-packages (from NanoPlot) (1.1
      Requirement already satisfied: python-dateutil in /usr/local/lib/python3.10/dist-packages (from Nano
      Collecting nanoget>=1.19.1 (from NanoPlot)
        Downloading nanoget-1.19.3.tar.gz (24 kB)
        Preparing metadata (setup.py) ... done
      Collecting nanomath>=1.0.0 (from NanoPlot)
        Downloading nanomath-1.3.0.tar.gz (18 kB)
        Preparing metadata (setup.py) ... done
      Requirement already satisfied: plotly>=5.4.0 in /usr/local/lib/python3.10/dist-packages (from NanoPl
      Requirement already satisfied: pyarrow in /usr/local/lib/python3.10/dist-packages (from NanoPlot) (1
      Collecting kaleido (from NanoPlot)
        Downloading kaleido-0.2.1-py2.py3-none-manylinux1_x86_64.whl (79.9 MB)
                                                79.9/79.9 MB 9.4 MB/s eta 0:00:00
      Collecting pandas>=1.1.0 (from NanoPlot)
```

Graphing structural variation versus incompatibility

Cross	Group working on comparison	Type of cross	Strength of Incompatibility	# Insertions	# Deletions	# Repeat expansions	# Repeat contractions	Total number of structural variants
SM x EGG	5	ExA	2	4	0	3	3	12
VA112 x EGG	6	ExA	2	2	1	1	2	8
SM x VA73	7	ExA	2	1	0	1	3	5
AL1 x SK	2	WxA	3	1	1	2	1	6
AR2 x VA73	8	WxA	3	1	0	2	2	5
MN4 x EGG	3	WxA	3	1	1	0	2	4
AR2 x VA112	4	WxE	1	2	0	0	1	3
WI4 x SM	1	WxE	1	1	0	0	1	3

Classroom participants

Fall 2023 and Spring 2024

Classroom sample size N=398:

- 68% female
- 52% Biology/Biotech Major
- 18% first-generation college student
- 15% transfer students

Evaluating Impact

- Student reported – post module survey
- Likert scales were used to assess impact
  - Conceptual learning
  - Interest gained
  - Confidence gained
- For statistical analysis neutral and negative answers were combined, and positive answers were combined
- Data were then modeled using a binomial distribution

1	2	3	4
Substantially	Moderately	Very little	Not at all

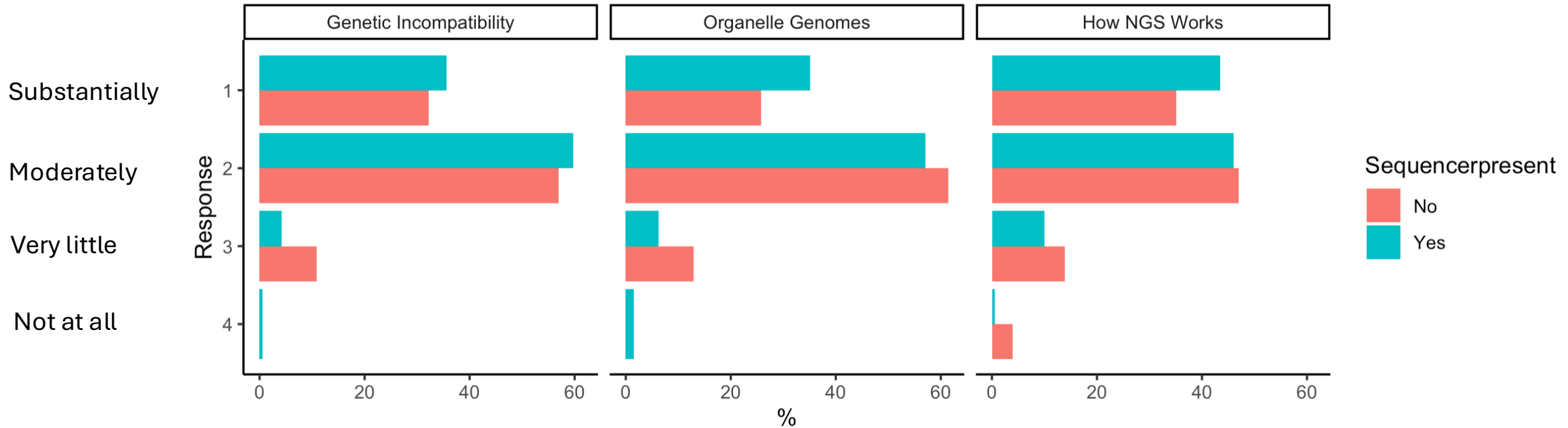| 1	2	3	4	5
Strongly agree	Agree	Neutral	Disagree	Strongly Disagree

# Sequence presence did not impact conceptual understanding

The module increased my conceptual understanding of...

- Module was successful in increasing conceptual understanding
- No significant impact of sequencer presence, though there is a positive trend

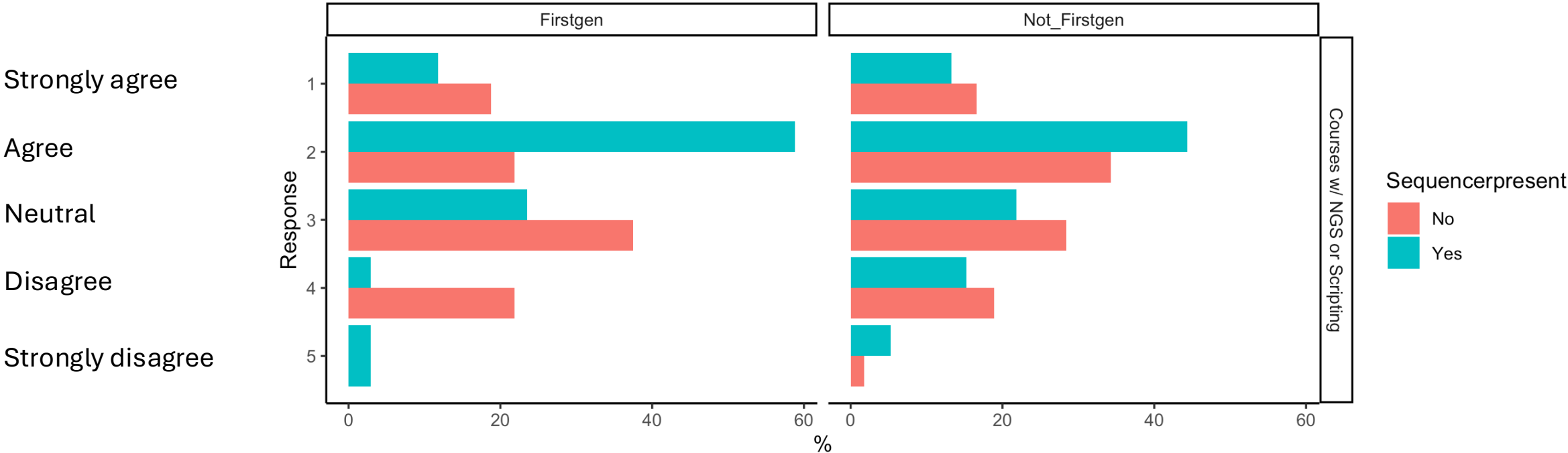# Sequencer presence had some impact on increased interest

Do what extent do you agree with the statement: The module has made me more interested in....
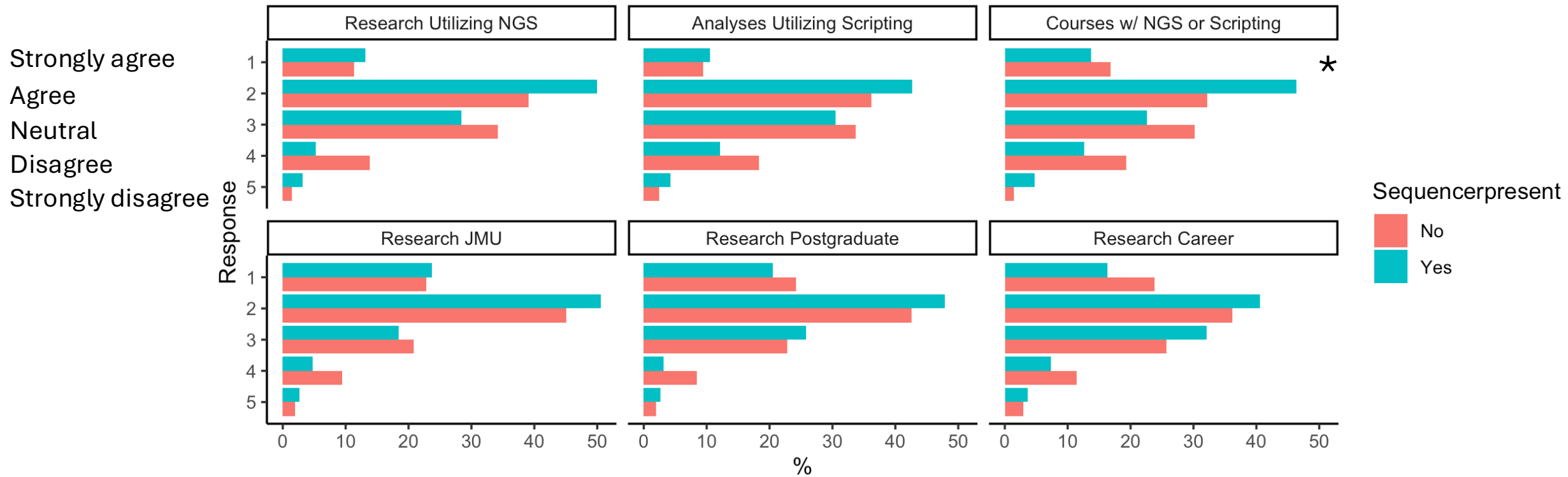
- Sequencer presence generally leads to more positive responses
  - This was significant for only one question

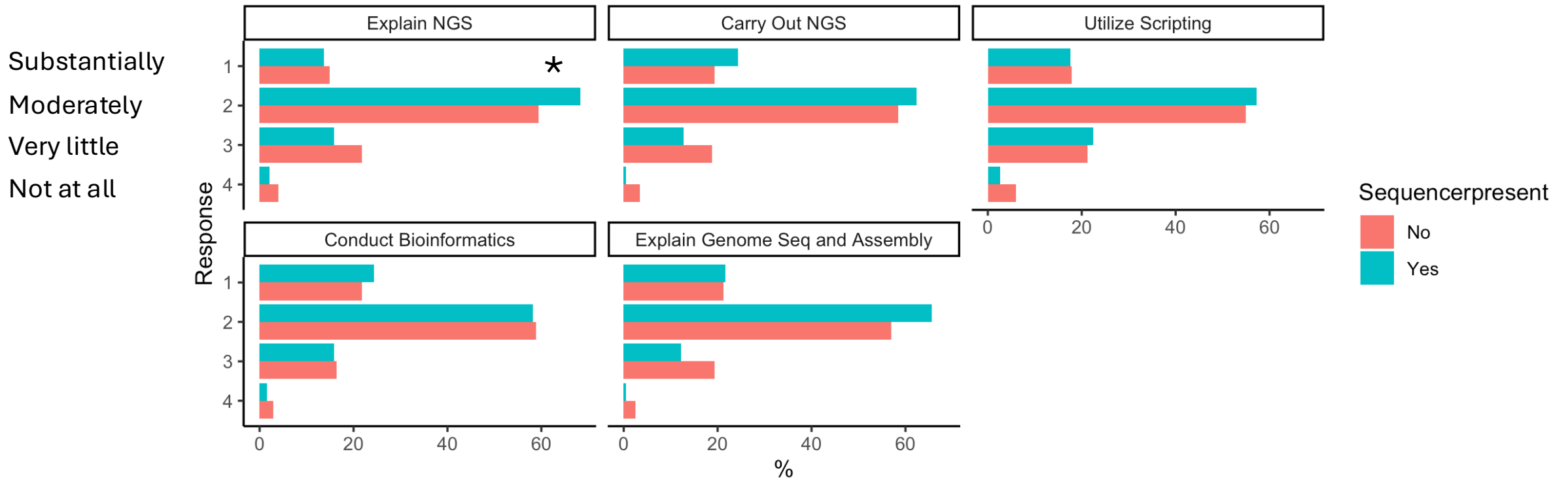# Sequencer presence had a greater impact on first-gen students

Impact of sequencer presence on increased interest was more pronounced for first-generation students

However, the opposite effect was seen for transfer students.

# Sequencer presence had some impact on increased confidence

The module increased my confidence in my ability to...

- Sequencer presence generally leads to more positive responses
- This was significant for only one question

Sequencer presence had a greater impact on first-gen students

- Impact of sequencer presence on increased confidence was more pronounced for first-generation students
- Seen for both ability to explain NGS as well as ability to utilize scripting
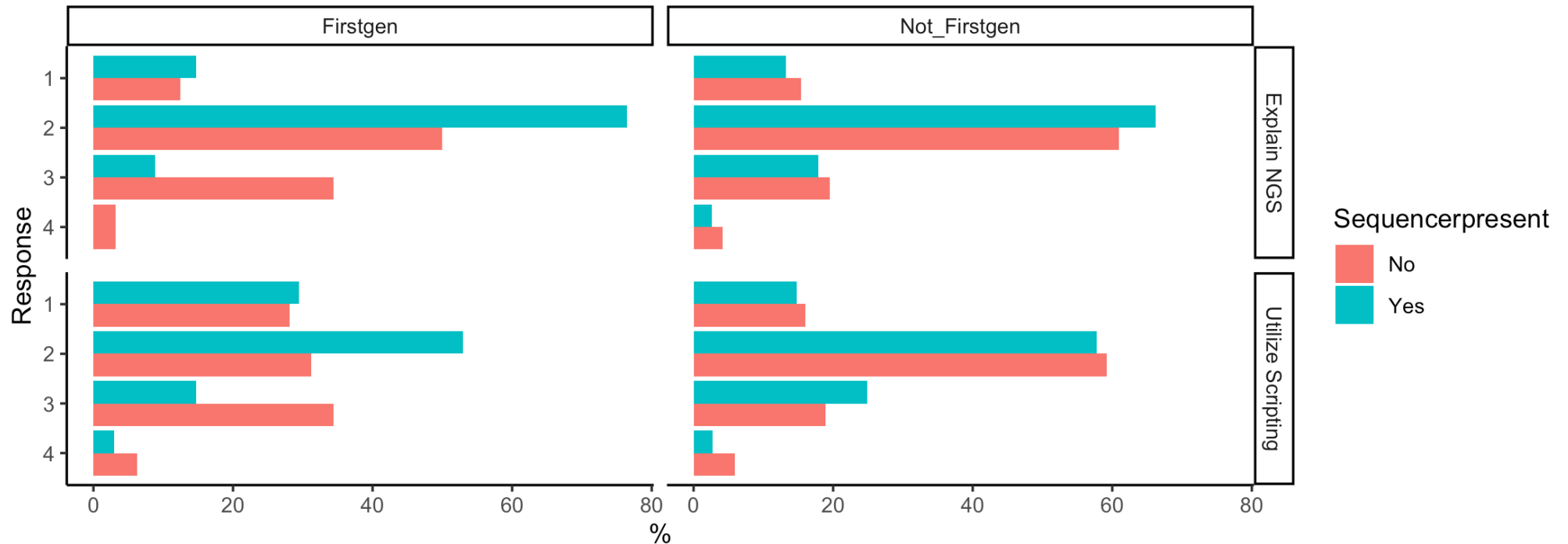
No significant interaction effect for transfer students

Takeaways

- The module overall was successful in increasing students conceptual understanding, interest, and confidence in NGS and command line scripting
- Sequence presence generally had a positive impact
  - The impact was more pronounced for first-generation students
  - Impact on transfer students was either negative or neutral
- Biology/biotech majors responded more positively than other majors (health science)
- We learned many lessons about what works and doesn't work
  - I would love to talk more about this later!

Acknowledgements

JMU
- Tyler Gandee
- Joe Harsh
- Caylin Murray
- Isobel Cobb
- Ray Enke
- Genetics Lab Instructors
  - Tim Bloss
  - Kristen Clermont
  - Susan Halsell
  - Kimberly Slekar
  - Marquis Walker

UVA
- Alfredo López-Camaal
- Laura Galloway