

OTU vs. ASV: Clash of the “Species”

**MICB425 Project 1
2018 Term 2**

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LEARNING OBJECTIVES

Students will be able to:

- Describe the strengths and weaknesses of iTag/amplicon based sequencing approaches
- Recognize the basic steps of amplicon data analysis
- Define OTU and ASV
- Identify differences between an OTU and an ASV pipeline

IN CONTEXT

- Who is there? • iTag/amplicon sequencing
- What could they be doing? • Metagenomics
- What are they trying to do? • Meta-transcriptomics
- What are they doing? • Proteomics

iTAG / AMPLICON SEQUENCING

- Highly targeted approach for analyzing genetic variation in specific genomic regions
- In-depth sequencing of a small piece of the genome
- Used to identify all* taxa in a complex microbial community

*Actual results may vary

BASIC WET LAB PIPELINE

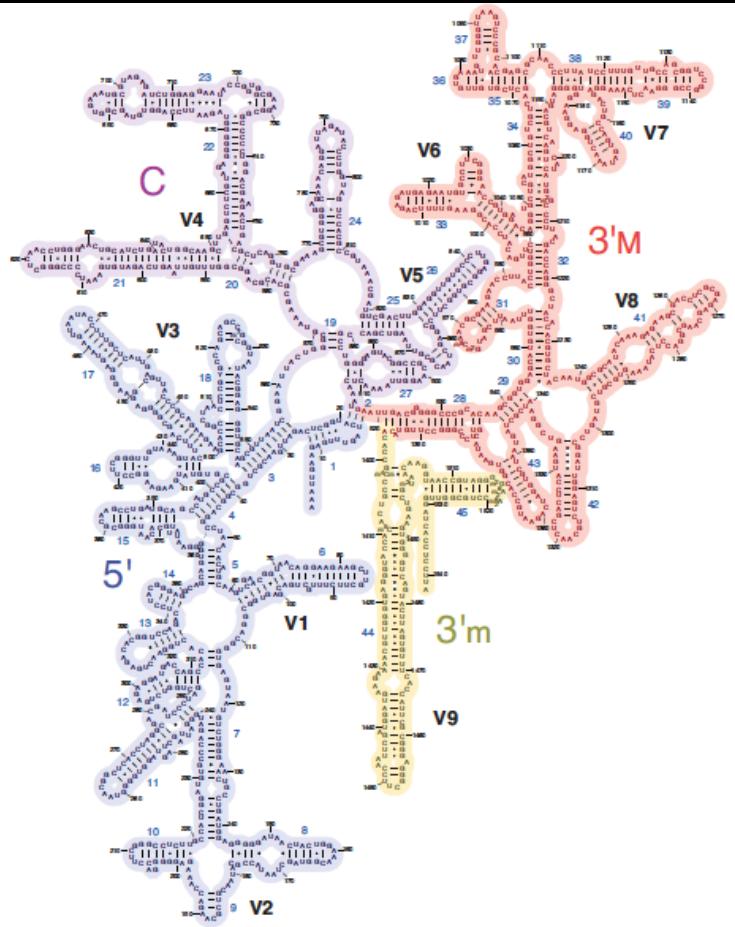
Complex sample of interest

Total genomic DNA extraction

PCR amplify target amplicon

Parallel sequencing of amplicon
(usually MiSeq or HiSeq)

CHOOSE YOUR AMPLICON



- Bacteria: 16S
V1-2, V3-4, V4, V4-5, V6-8
- Archaea: 16S, 18S
- Fungi: ITS1, ITS2

Pros

- Reasonably accurate sample of microbes in a community
- Cheap! 100s of communities can be run for ~ \$1000
- Helps inform which samples are of interest for further analyses (like metagenomics)

Cons

- Biases from primer choice, PCR, gene copy number, etc.
- It's all *relative* abundance
- Alive or dead?
- But what are the microbes doing?



So you've done amplicon sequencing, now
what?

mothur and QIIME2

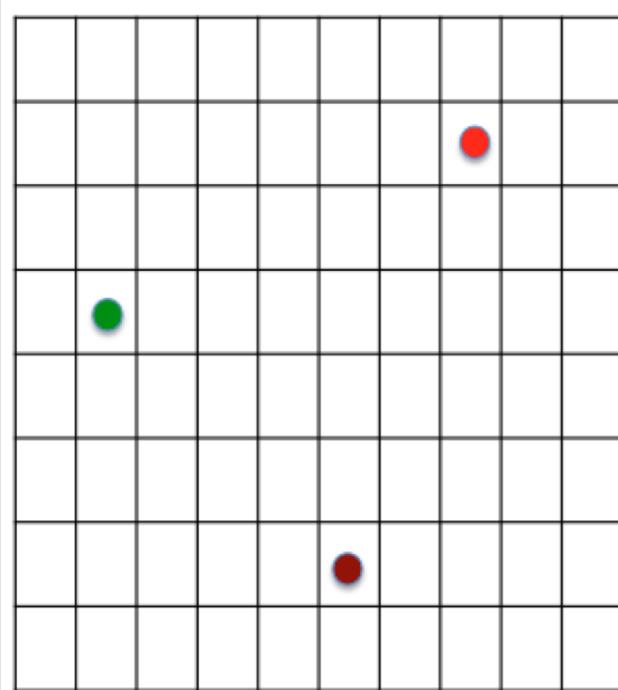
- Programs specifically designed to process amplicon (and in particular, 16S) sequencing data
- Which do you choose?

THE UNDERLYING CHOICE

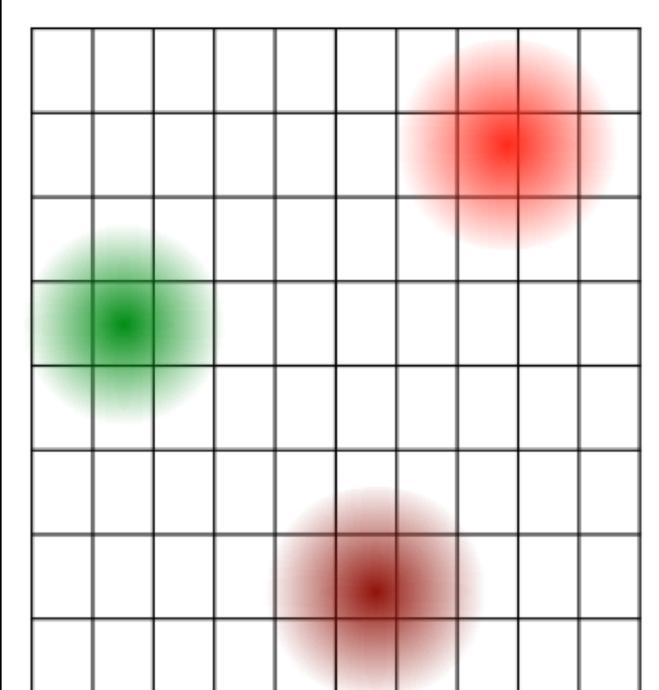
Operational taxonomic units (OTUs)

vs.

Amplicon sequence variants (ASVs)

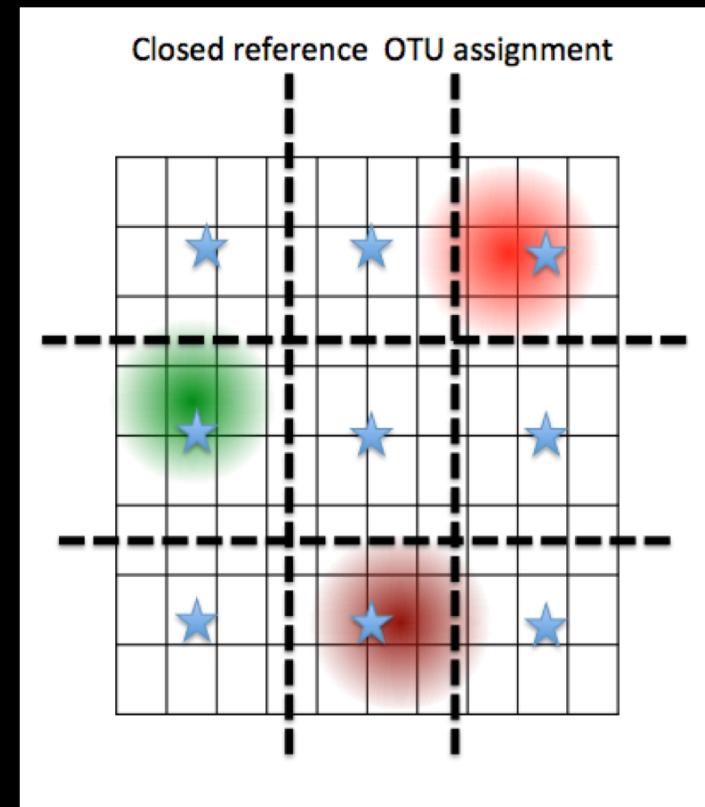
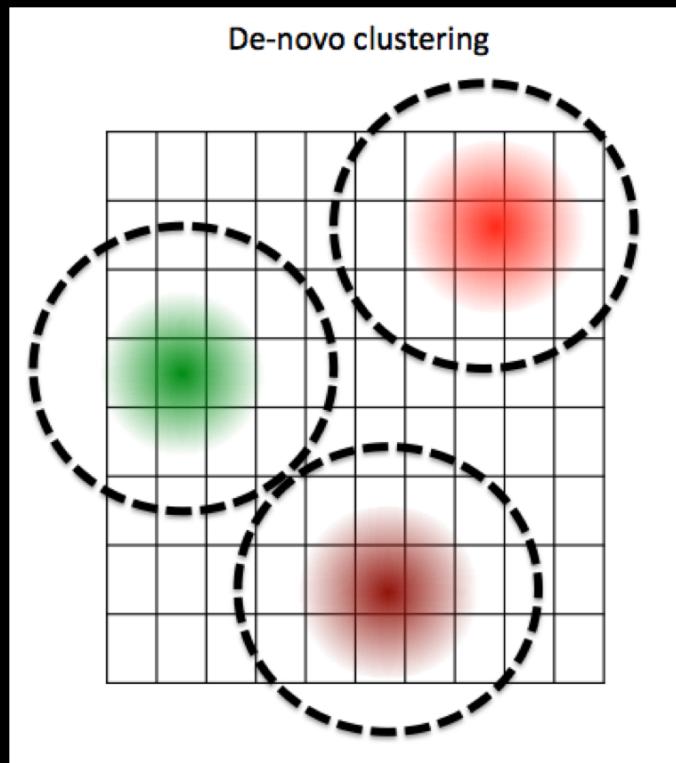


True sequences

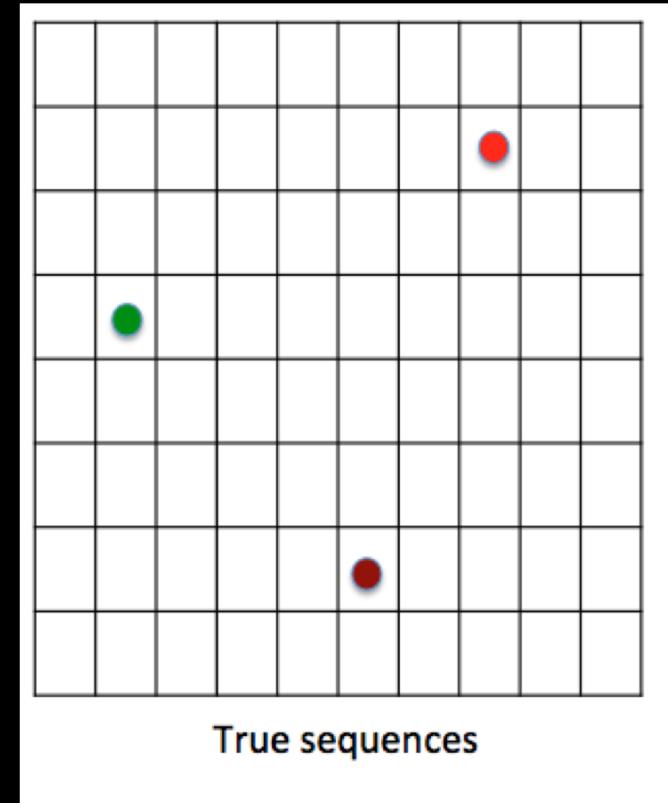
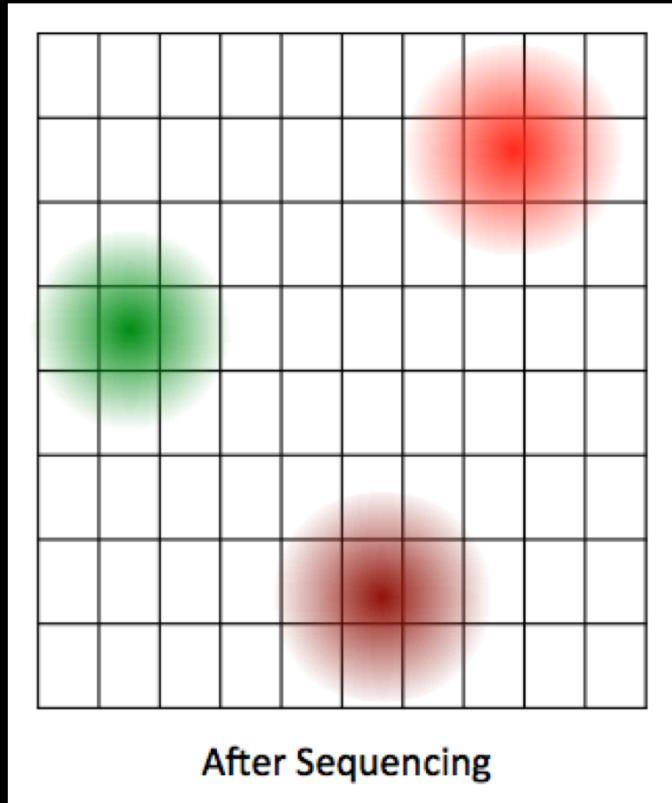


After Sequencing

OTU CLUSTERING



ASV DE-NOISING



OTU

- Most previous literature
- Keeps more data though not all of this data represents “real” taxa in the community
- Uses representative sequence of each OTU to determine taxonomy
- mothur or QIIME

ASV

- New 2017
- Discards more data potentially biasing relative abundances toward low error-prone sequences
- Treats each ASV as a “species”
- QIIME2

PROJECT 1

- Compare the same data processed as OTUs and ASVs
- Same samples, same raw sequences
- Do you get the same answers to biologically relevant questions using data processed as OTUs vs. ASVs?

TIMELINE

- March 7: OTU vs. ASV lecture
- March 9: Intro to Saanich lecture + start group work
 - You should explore the data and choose your taxon of interest by end of day
- March 12: Group work day
- March 14: Intro to statistics lecture + more group work
- March 16: Final group work day
- March 28: Soft deadline for draft to receive feedback
- April 25: Final reports due with final portfolios

MATERIAL

- In Module_03/Project1
 - Instructions
 - Data!
 - Detailed pipelines for mothur and QIIME2
 - Phyloseq objects of OTU/ASV table, taxonomy, and metadata
 - A mock report to give you some ideas for figures and statistical tests



**NOT SURE IF I HATE GROUP
PROJECTS**

OR JUST HATE PEOPLE

WHY GROUP WORK?

- Communication and teamwork skills
- Content and concept reinforcement through discussion and peer-teaching
- Pool diverse expertise, knowledge and skills
- Accomplish more as a team than can individually

- It's how scientific research happens
- It's how business happens

EVIDENCE FOR GROUP WORK HERE AT UBC

Gaudet AD *et al.* 2010. Small-group learning in an upper-level university biology class enhances academic performance and student attitudes toward group work. PLoS One 5(12): e15821.

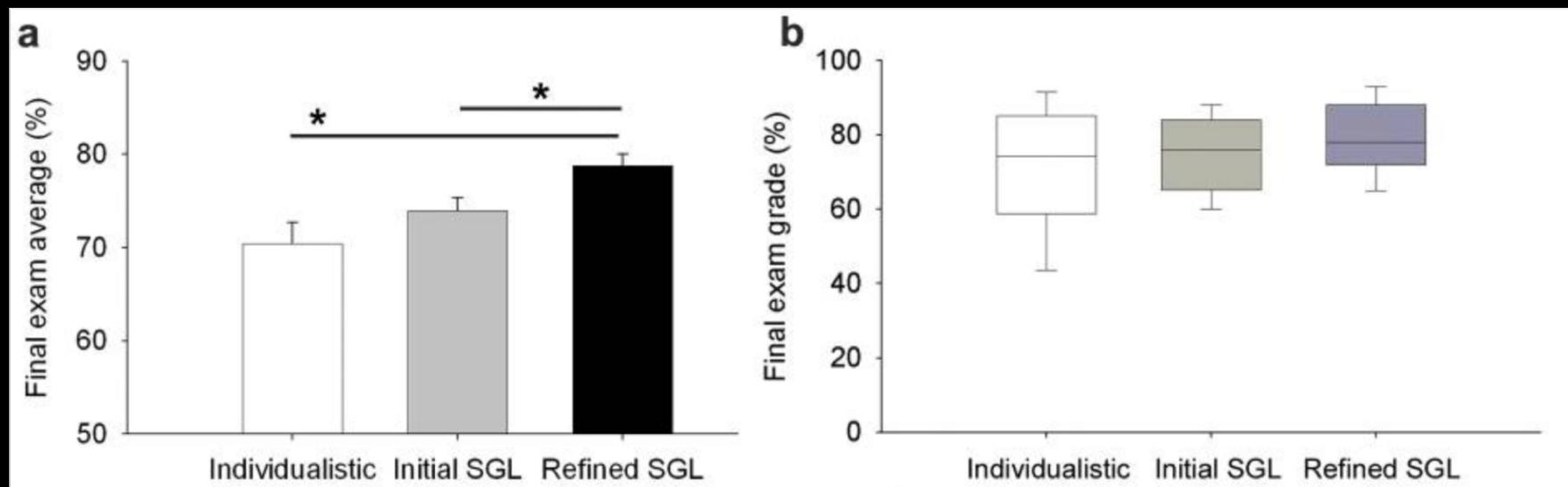
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3012112/>

- Neurobiology course at UBC!
- Compare sections where students completed all quizzes and in-class assignments in groups vs. individually

EVIDENCE

Group work improves student learning

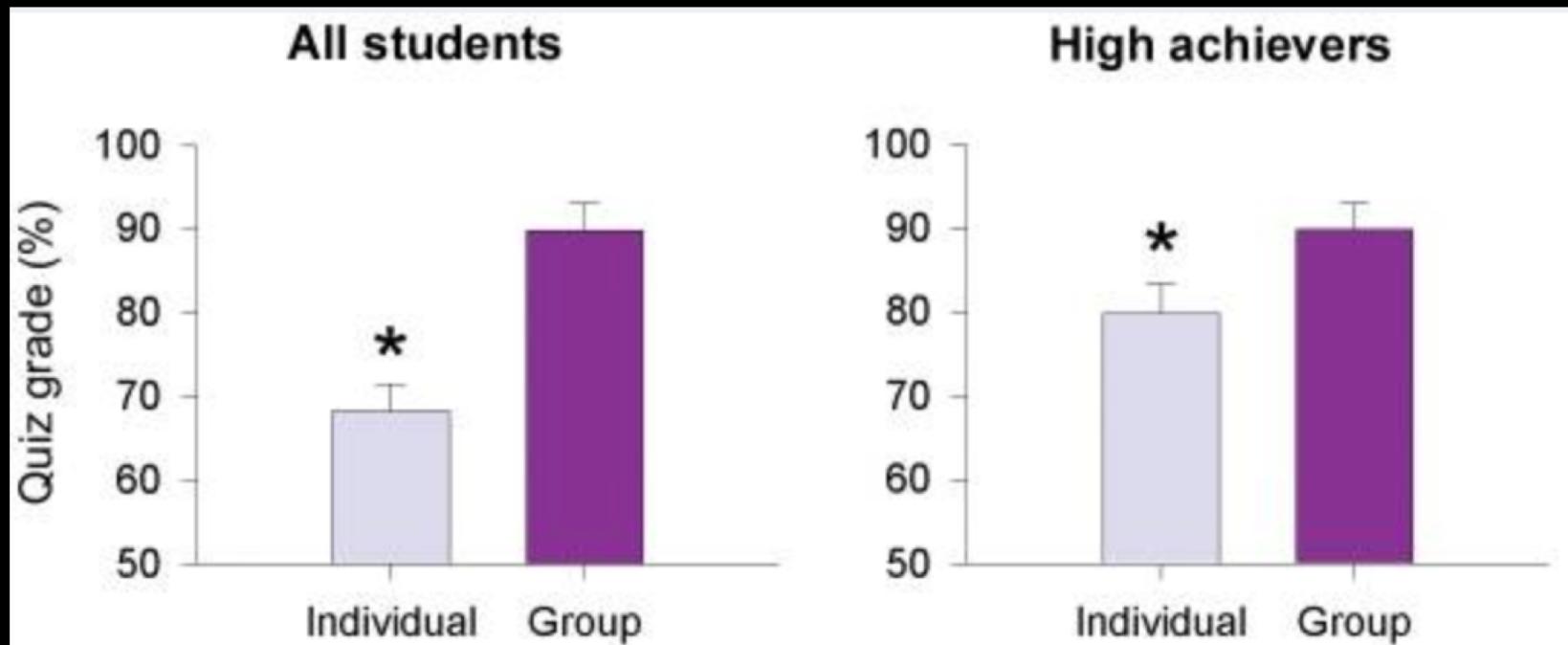
Average grades within sections Individual student grades



SGL = small-group learning

EVIDENCE

Highest achieving students still benefited



DON'T BELIEVE ME? BELIEVE SCIENCE!

- Bransford, J.D., Brown, A.L., and Cocking, R.R. (Eds.) (1999). *How people learn: Brain, mind, experience, and school*. Washington, D.C.: National Academy Press.
- Bruffee, K. A. (1993). *Collaborative learning: Higher education, interdependence, and the authority of knowledge*. Baltimore, MD: Johns Hopkins University Press.
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- Davidson, N., & Major, C. H. (2014). Boundary crossing: Cooperative learning, collaborative learning, and problem-based learning. *Journal on Excellence in College Teaching*, 25 (3&4), 7-55.
- Dees, R. L. (1991). The role of cooperative leaning in increasing problem-solving ability in a college remedial course. *Journal for Research in Mathematics Education*, 22(5), 409-21.
- Gokhale, A. A. (1995). Collaborative Learning enhances critical thinking. *Journal of Technology Education*, 7(1).
- Johnson, D.W., Johnson, R.T., and Smith, K.A. (2014). Cooperative learning: Improving university instruction by basing practice on validated theory. *Journal on Excellence in College Teaching* 25, 85-118.
- Jones, D. J., & Brickner, D. (1996). Implementation of cooperative learning in a large-enrollment basic mechanics course. *American Society for Engineering Education Annual Conference Proceedings*.
- Love, A. G., Dietrich, A., Fitzgerald, J., & Gordon, D. (2014). Integrating collaborative learning inside and outside the classroom. *Journal on Excellence in College Teaching*, 25(3&4), 177-196.
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- Springer, L., Stanne, M. E., & Donovan, S. S. (1999). Effects of small-group learning on undergraduates in science, mathematics, engineering, and technology: A meta-analysis. *Review of Educational Research*, 96(1), 21-51.
- Uribe, D., Klein, J. D., & Sullivan, H. (2003). The effect of computer-mediated collaborative learning on solving ill-defined problems. *Educational Technology Research and Development*, 51(1), 5-19.
- And more...

GROUP ASSESSMENT

- CATME + contributions to the group report
- This will account for 20% of your individual marks for the project

For example:

- If your group report received a 90% and you were an exemplary group member, your mark would be
 $0.9 * 0.8 + 0.2 = 0.92$
- If you were a very poor group member but still contributed to the project in some way, it would be
 $0.9 * 0.8 + 0 = 0.72$
- Those who do not contribute to their group will be asked to complete the report independently

GROUPS

Do you want to have the same groups for
Project 1 and 2?

Or mixup all groups between projects?

PROJECT 1 QUESTIONS

1. How does microbial community structure change with depth and oxygen concentration?
 - a. Alpha-diversity
 - b. Taxa presence and abundance
2. Does your taxon of interest *significantly* differ in abundance with depth and/or oxygen concentration?
3. Within your taxon, what is the richness (number of OTUs/ASVs)?
4. Do the abundances of OTUs/ASVs within your taxon of interest change *significantly* with depth and/or oxygen concentration?
5. Are the answers to the above the same using mothur and QIIME2 processed data?