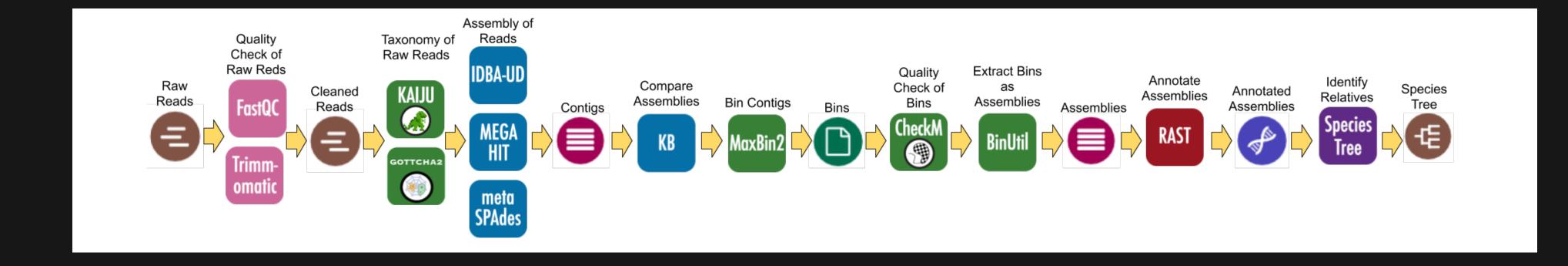
Assembling Knowledge of Metagenome Assembled Genomes (MAGs) through Annotation and KBase



We used a combination of primary literature, pre/post quizzes, bioinformatics attitude surveys, and KBase narratives to learn how students perform and analyze fundamental metagenome assembled genomes (MAGs).



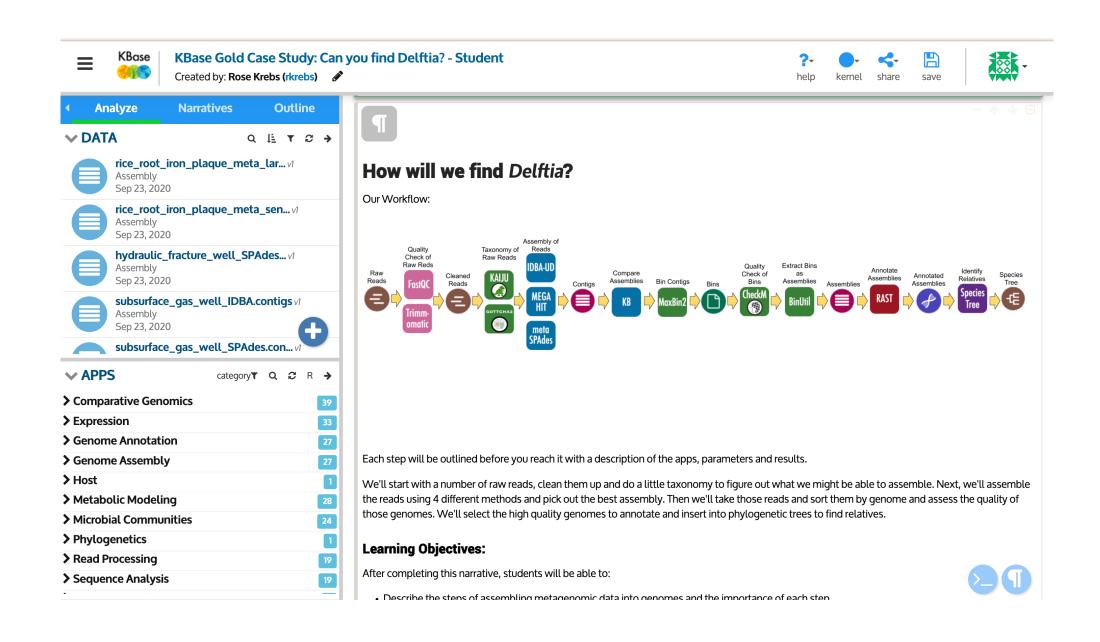








FIGURE 1: KBase Narrative case studies engage students in assembly and binning of sequences for taxonomic analyses.

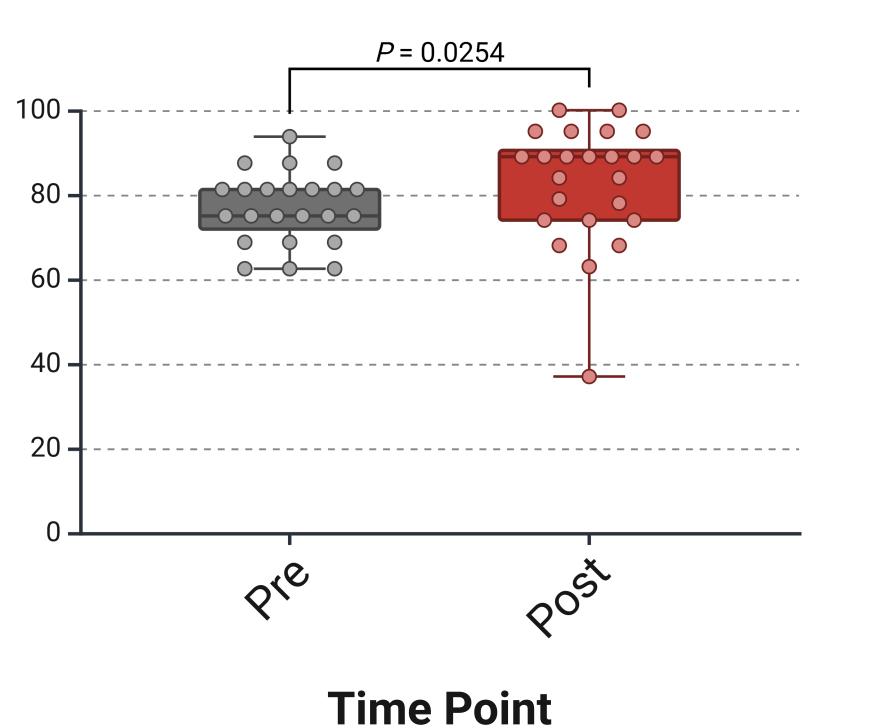


ere, we report the development of stable switchgrass-fermenting enrichment cultures maintained at various residence times and moderately high (55 °C) temperatures. Anaerobic microbiomes derived from a digester inoculum were incubated at 55 °C and fed semi-continuously with medium containing 30 g/L mid-season harvested switchgrass to achieve residence times (RT) of 20, 10, 5, and 3.3 days. Stable, time-invariant cellulolytic methanogenic cultures with minimal accumulation of organic acids were achieved for all RTs. Fractional carbohydrate solubilization was 0.711, 0.654, 0.581 and 0.538 at RT = 20, 10, 5 and 3.3 days, respectively, and glucan solubilization was proportional to xylan solubilization at all RTs. The rate of solubilization was described well by the equation $r = k(C - C_0 f_r)$, where C represents the concentration of unutilized carbohydrate, C_0 is the concentration of carbohydrate (cellulose and hemicellulose) entering the bioreactor and f_r is the extrapolated fraction of entering carbohydrate that is recalcitrant at infinite residence time. The 3.3 day RT is among the shortest RT reported for stable thermophilic, methanogenic digestion of a lignocellulosic feedstock. 16S rDNA phylotyping and metagenomic analyses were conducted to characterize the effect of RT on community dynamics and to infer functional roles in the switchgrass to biogas conversion to the various microbial taxa. Firmicutes were the dominant phylum, increasing in relative abundance from 54 to 96% as RT decreased. A *Clostridium clariflavum* strain with genetic markers for xylose metabolism was the most abundant lignocellulose-solubilizing bacterium. A Thermotogae (Defluviitoga tunisiensis) was the most abundant bacterium in switchgrass digesters at RT = 20 days but decreased in abundance at lower RTs as did multiple Chloroflexi. Synergistetes and Euryarchaeota were present at roughly constant levels over the range of RTs examined.

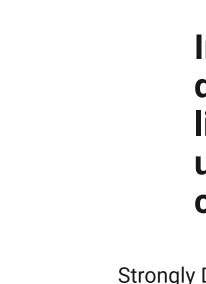
Participants learn about metagenome assembled genomes (MAGs) through a step-by-step KBase Narrative that introduces trimming, assembly, binning, annotation, and phylogenetic trees. Next, participants are challenged to find *Delftia acidovorans* MAGs (above).

Scholars annotate texts collaboratively to learn about MetaBAT2 (Kang *et al.* 2019 **above**) and tools for metagenomic assembly. Two articles discuss the application of metagenomic assembly and binning (Ling *et al.* 2018 and Mason *et al.* 2014).

FIGURE 3: Students complete pre and post quizzes about MAGs and metagenomics.







Count -

Students complete **multiple-choice pre** (n=24) and **post** (n=23, fall 2021, fall 2022, and fall 2023) **quizzes** aligned with the learning objectives and addressing concepts from the articles and KBase narratives. Questions include analyses, definitions, and the purpose of tools (*e.g.*, MetaBat2 and CheckM). Pre/post differences are ns at this time.

Participants complete **post surveys** with questions about their confidence with **bionformatics tools** and how they approached annotation and web-based computational assignments.



PRESENTER



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Students annotate articles bioinformatics the and to tools applications of MAGs.

1	Assignment information
ł	Due Fri Oct 18, 2024 5:00 pm EDT
	We will read a series of articles in the BIT 477/577 Metagenomics course exposing you to current trends in metagenomics and analyses microbial communities. Each person will be required to provide at lea ten (10) meaningful (relevant, thoughtful, and specific) annotatio of each article discussed by the day the article is presented in class. Th activity will allow us to have productive discussions on the content. Go beyond superficial comments: connect information, highlight limitatio link to other studies, including tags, and respond to others!
	Part 1 of 1: Development and characterization of stable anaerobic thermophilic methanogenic microbiomes fermentin switchgrass at decreasing residence times Biotechnology for Biofuels and Bioproducts Full Text Entire document

FIGURE 4: Students share their thoughts on how they approach computational challenges.

Indicate the degree to which you agree or disagree with the following statements. - I like bioinformatics when using tools with user-friendly web-interfaces but do not like command-line style analysis.

y Disagree	Disagree	Neutral	Agree	Strongly Agree
1	6	5	4	8

ACKNOWLEDGEMENTS

The study was approved by the **NCSU IRB protocol # 20309.** We are grateful to the students for their patience and commitment to learning and analyzing complex datasets. We would like to thank the **KBase Education Community, especially Ellen Dow, Elisha WC, and the summer 2020** education group, for helpful tips and productive conversations. CCG was supported by the North Carolina State Biotechnology Teaching Program (BIT). The design, implementation, and dissemination of educational case studies to learn about highthroughput technologies is part of the **NSF-RCN HITS** (award #1730317).