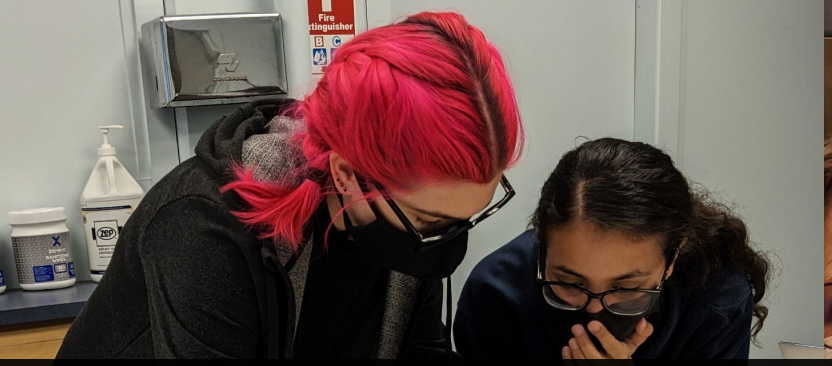
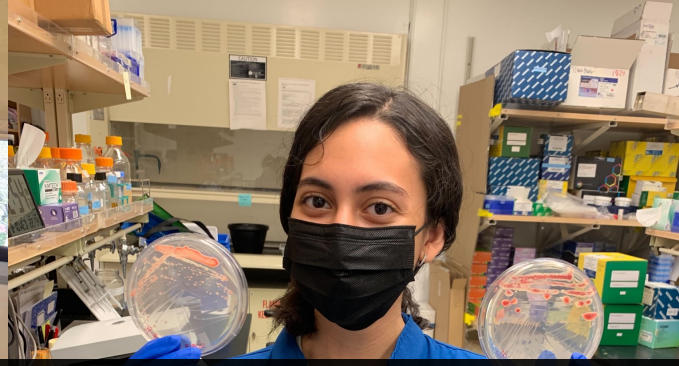
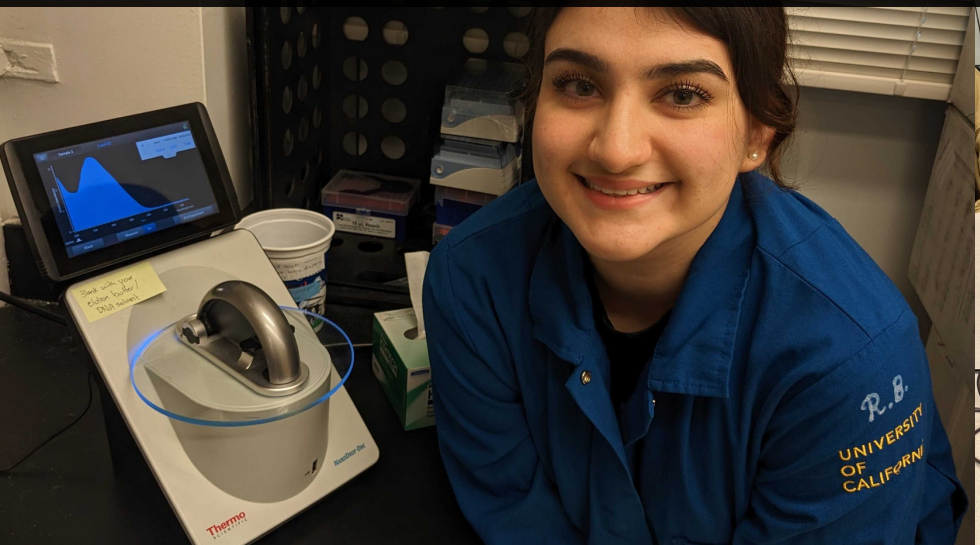
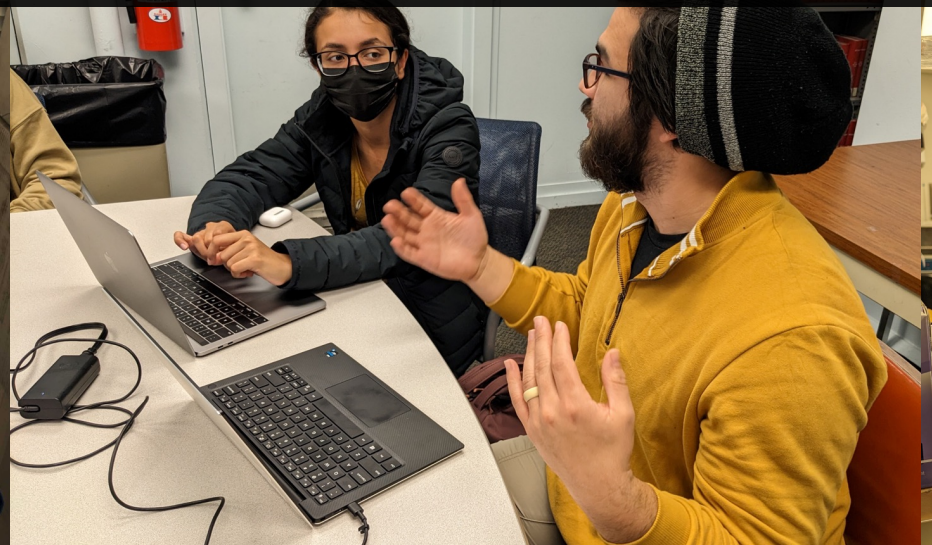




Integrating Undergraduate Researchers into Genome Analysis of Bacterial Culture Collections

Tiffany Lowe-Power

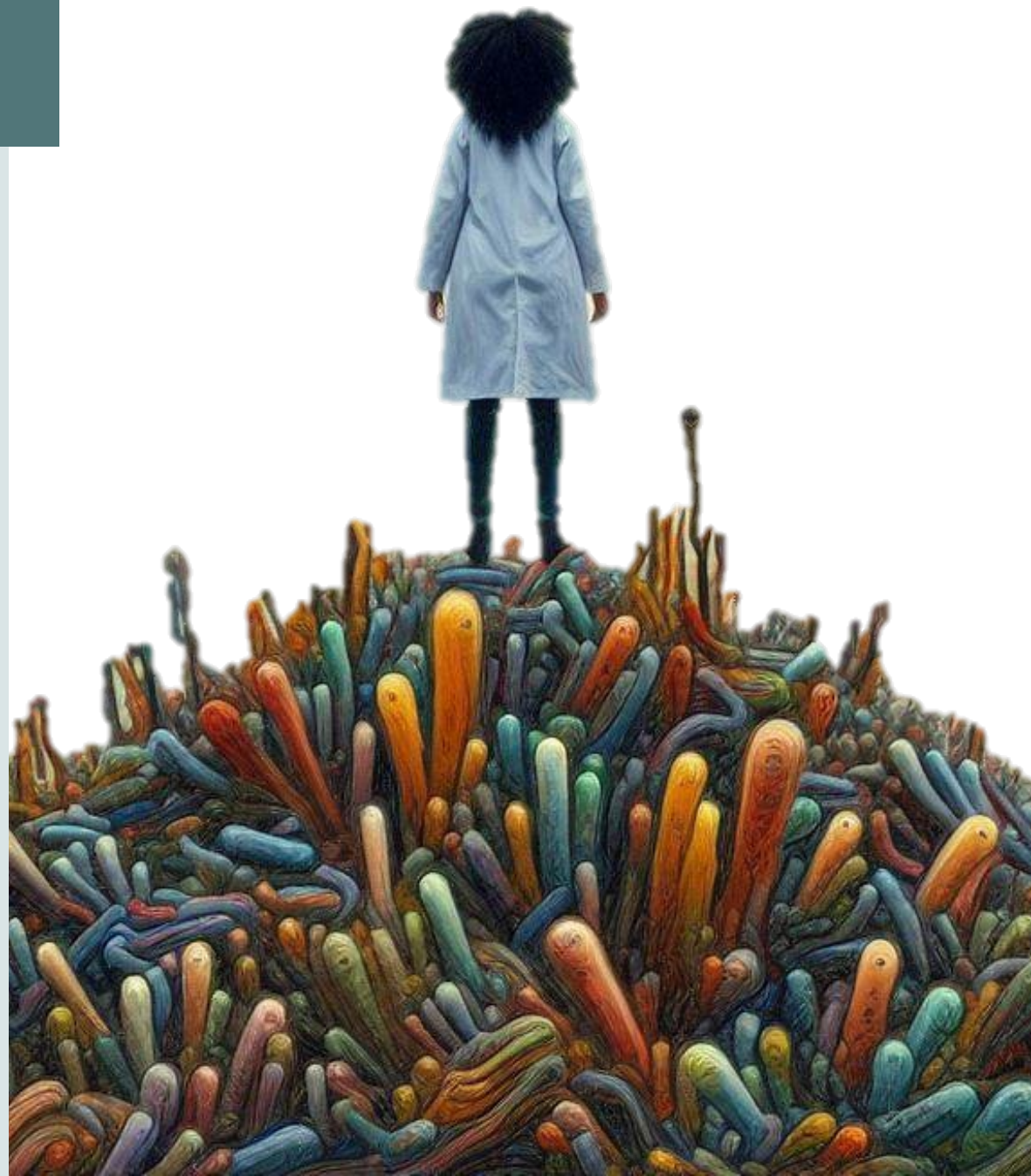
Asst Prof, UC Davis Plant Pathology


Take a Moment to Reflect:

How did you become a researcher?

- Role Models?
- Mentors?
- Research Opportunities?
- Identity as a Scientist?



My Story of Privilege and Opportunity:

- **Mentor 1** (Sam Parks): High School Bio Teacher who joined a PhD program
  - Connected me to a senior year research opportunity in an environmental micro lab – **identity as a researcher**
- **Mentor 2** (Dr. Leavey): Undergrad Bio Lecturer encouraged me to apply to NSF research experiences for undergrads (REUs).
  - Participated in 2 cohort opportunities (**network!**) with programming to **demystify hidden curriculum** of academia

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- **My aspirational goal:**
  "I want become a professor in order to train the next generation of Scientists"

Outline:

- **Cohort-based undergraduate research experiences (CUREs):** A semi-scalable approach to training the next generation of scientists
- Development of the *Ralstonia* genomics CURE
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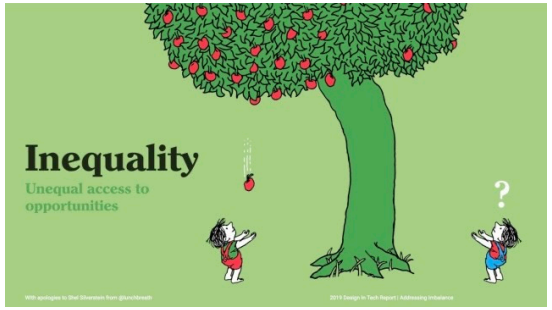
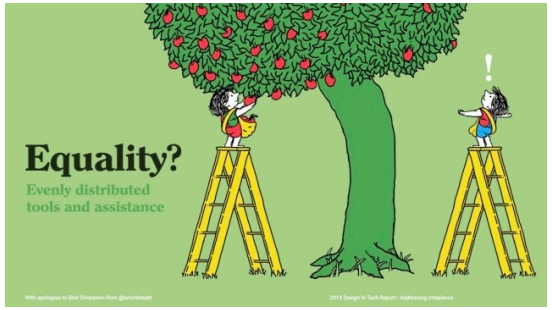
- Contributions of *Ralstonia* genomics CURE to scientific knowledge

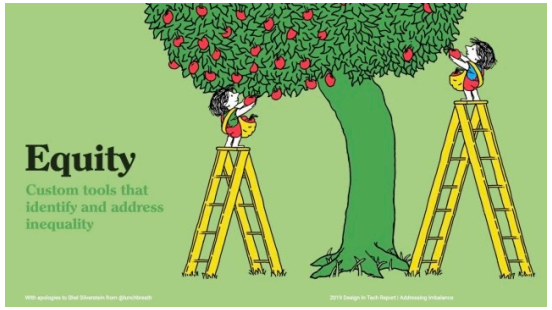
How to equitably train the next generation of scientists?



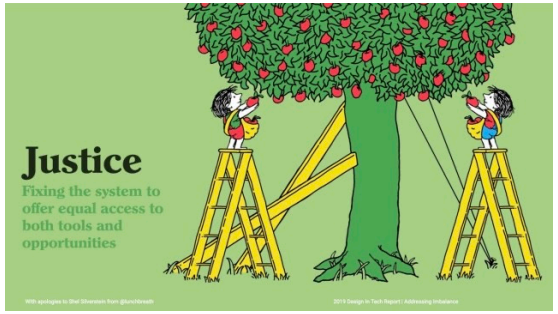
Rely on undergraduates to contact labs



Research requirement for all students



-Paid research
-Peer networks



How to equitably train the next generation of scientists?

Cohort-based Undergrad Research Experiences (**CUREs**)*

Elements of a CURE:

- Learn a variety of research skills
- Uncover the unknown through real research
- Learn, Apply, REPEAT
- Communication & collaboration

* CURE is usually “Class-based”, but I am avoiding the paperwork of formalizing my CURE…

Examples of CUREs

SEA PHAGES

Students **isolate bacteriophages** against BSL-1 pathogens. **Sequence bacteriophage genomes** and deposit on NCBI

tiny earth

Small World Initiative
crowdsourcing antibiotic discovery

Students isolate bacteria that produce **antibiotics** that inhibit BLS-1 pathogens. Identify strains by 16S rRNA sequencing and identify the antibiotic (chemistry CURE)

BASIL™

Students **assign function to proteins** by investigating protein structure data and purifying/assaying proteins

iGEM

Student (competitors) **genetically engineer** microbes for synthetic biology goals

Ingredients for a fruitful and sustainable CURE

Friendly to beginners who are learning by making mistakes

- Safe
- Not too expensive
- Doesn't require specialized technical skill

Scalable Projects

- Standardized methods on a parallel research objective/question
- Students get ownership over their work, but they can learn from each other.
- Doesn't stretch the mentors too thin

Useful to research community

- Connection to widely used databases (NCBI, PDB, etc)
  data generation or data analysis
- Published papers… if time/energy/funding permits

SEA PHAGES tiny earth Small World Initiative crowdsourcing antibiotic discovery BASIL™ iGEM

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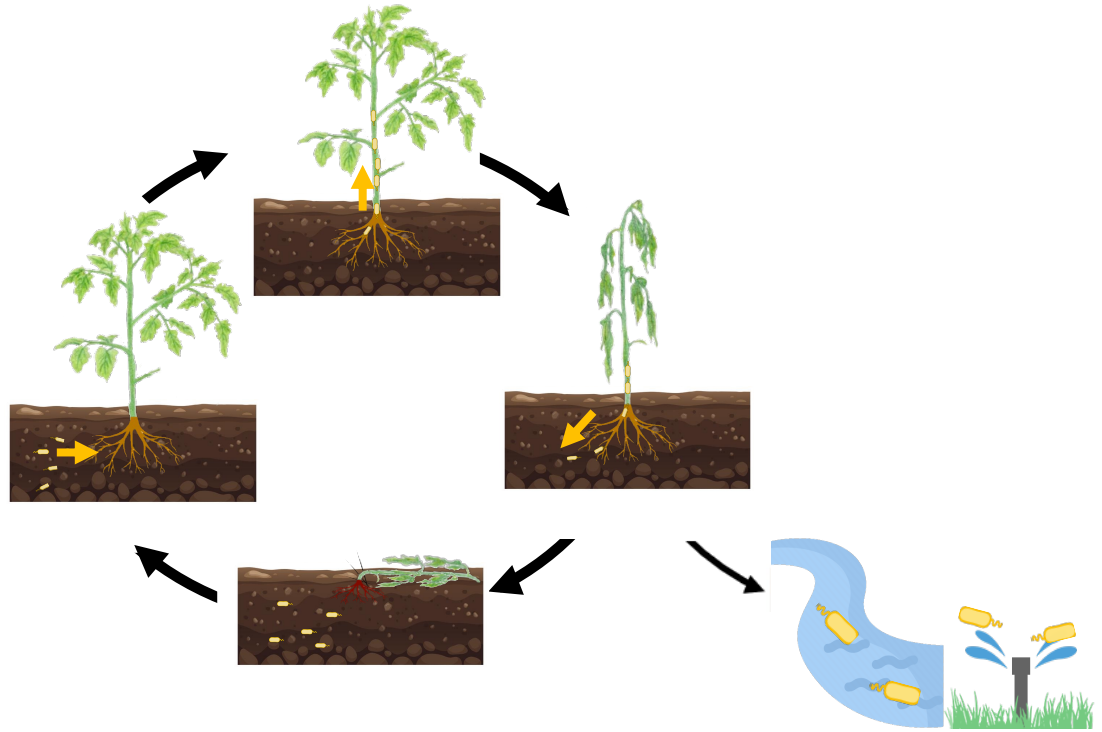
How can I "CURE" my research interests?

I like understanding **bacterial behavior** in **complex environments** and how **evolution** influences these behaviors.

My focal bacteria: ***Ralstonia solanacearum* species complex**



What gaps in knowledge about *Ralstonia* are CURE-able??

Ivan Buddenhagen:

The Conceptual Problem

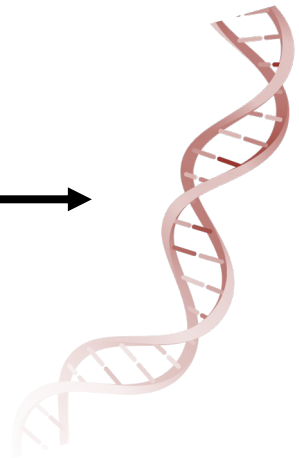
'Bacterial wilt' is two words but one conceptual entity. *Pseudomonas solanacearum*, likewise, is a single epithet. Although the literature is replete with 'biotypes' and 'strains', it generally treats 'wilt' as if there is **one** disease and **one** pathogen, created, I presume, by God, all at once, everywhere. We still live in a creationist, Linnean, descriptive, recording of 'static' phenomena which occur around us in our approach to our disease research—no matter whether we are generalists, biochemists, or molecular geneticists.

The reality, of course, is very different. There are many bacterial wilts and there are many '*Pseudomonas solanacearums*'. They have originated and evolved in widely different places and they have different capabilities with both native flora and introduced hosts, and presumably with different soils and environmental conditions.

Bacterial Wilt in the South Pacific (1985 conference proceedings)

Understanding the many faces of *Ralstonia:*
Training junior scientists

Ralstonia genomics CURE



Whole Genome Sequencing

Genome Assembly, Phylogenomics, Exploration

KBase

Graphical User Interface (Jupyter notebook) + Cloud server

Lowe-Power *et al.* BioRxiv https://github.com/lowepowerlab/Ralstonia_Global_Diversity

My recipe for teaching the *Ralstonia* genomics CURE:

Diverse *Ralstonia* isolates

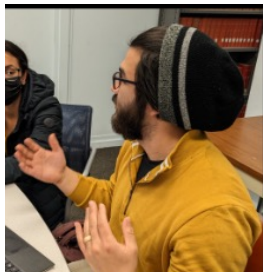
Cost-effective whole genome sequencing for bacteria

SeqCoast GENOMICS

Short read sequencing, Illumina	Price
Short reads; small 200 Mbp/1.3 Million reads	$77.00
Short reads; medium 400 Mbp/2.7 Million reads	$101.00

A Network

Near-peer role models (Grad Students)



Matt Cope-Arguello

Vienna Elmgreen

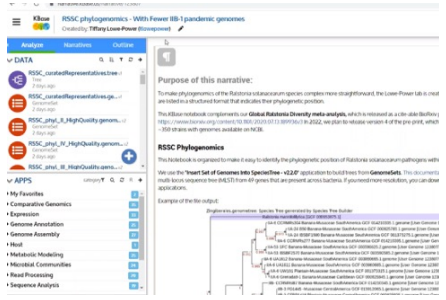
Peers

Me

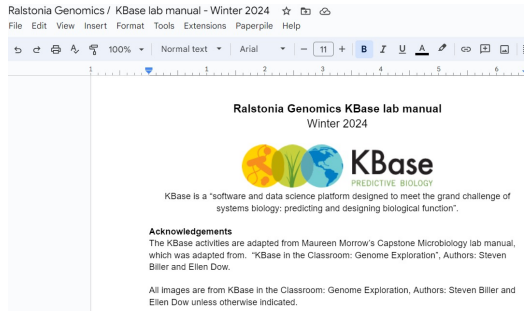
"Co-Working Sessions"

A cozy conference room

KBase



Bioinformatic Lab Manual on Google Docs



Adapted from Maureen Morrow, Steven Biller and Ellen Dow

The *Ralstonia* genomics CURE Experience:

A “Flipped” Research experience?

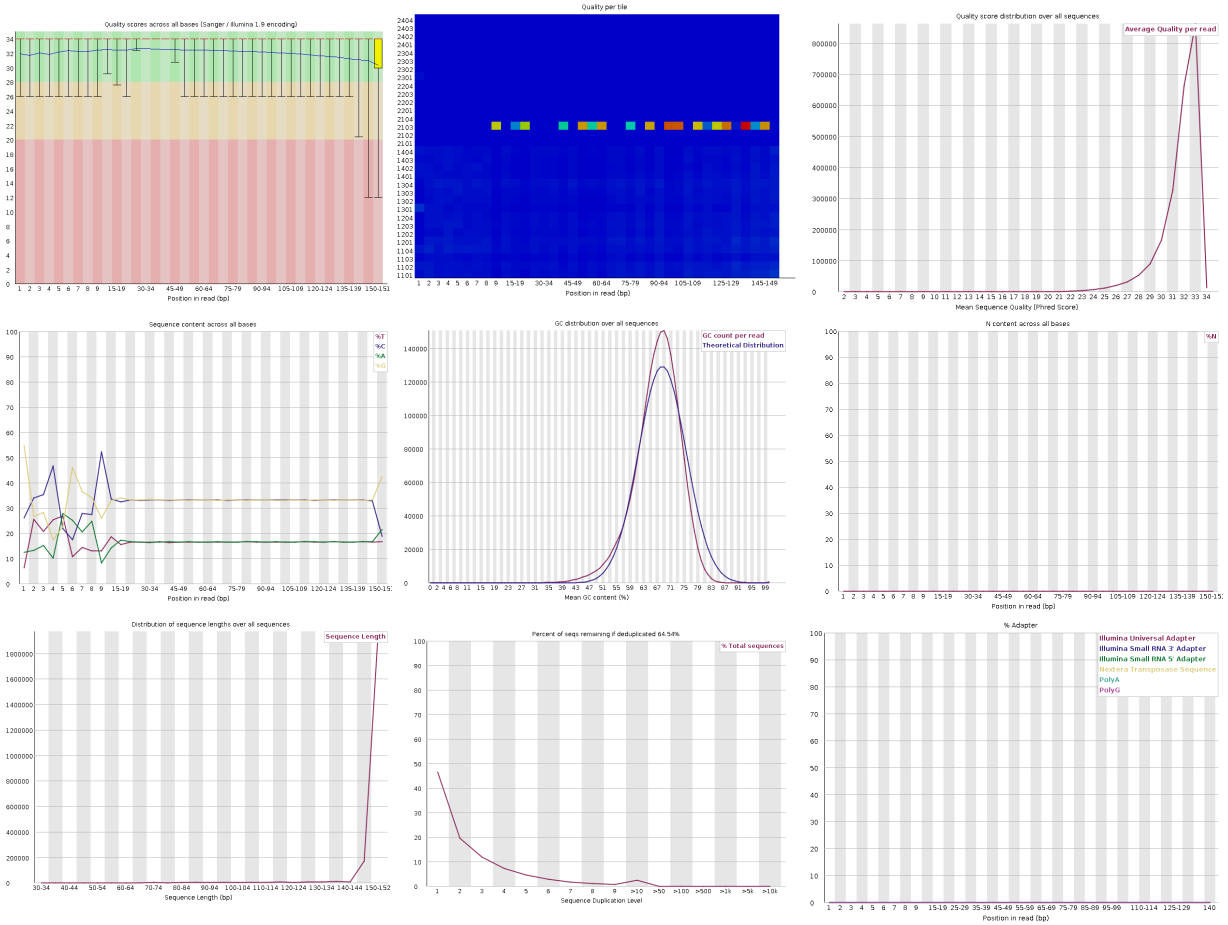
Dive into **data analysis *before* mastery** of any technical skills

Not everyone wants a career as a researcher, and that is okay!

The *Ralstonia* genomics CURE Experience:

Week 1:

Run FastQC to determine quality of the Illumina sequencing data



"… What does this graph mean?"

"What are the **axes' labels**?"

"What does the **documentation** say?"

"**Ask your cohort peer** to teach you what we just taught them"

"What does **Google** say?"

"What do **you think** it means?"

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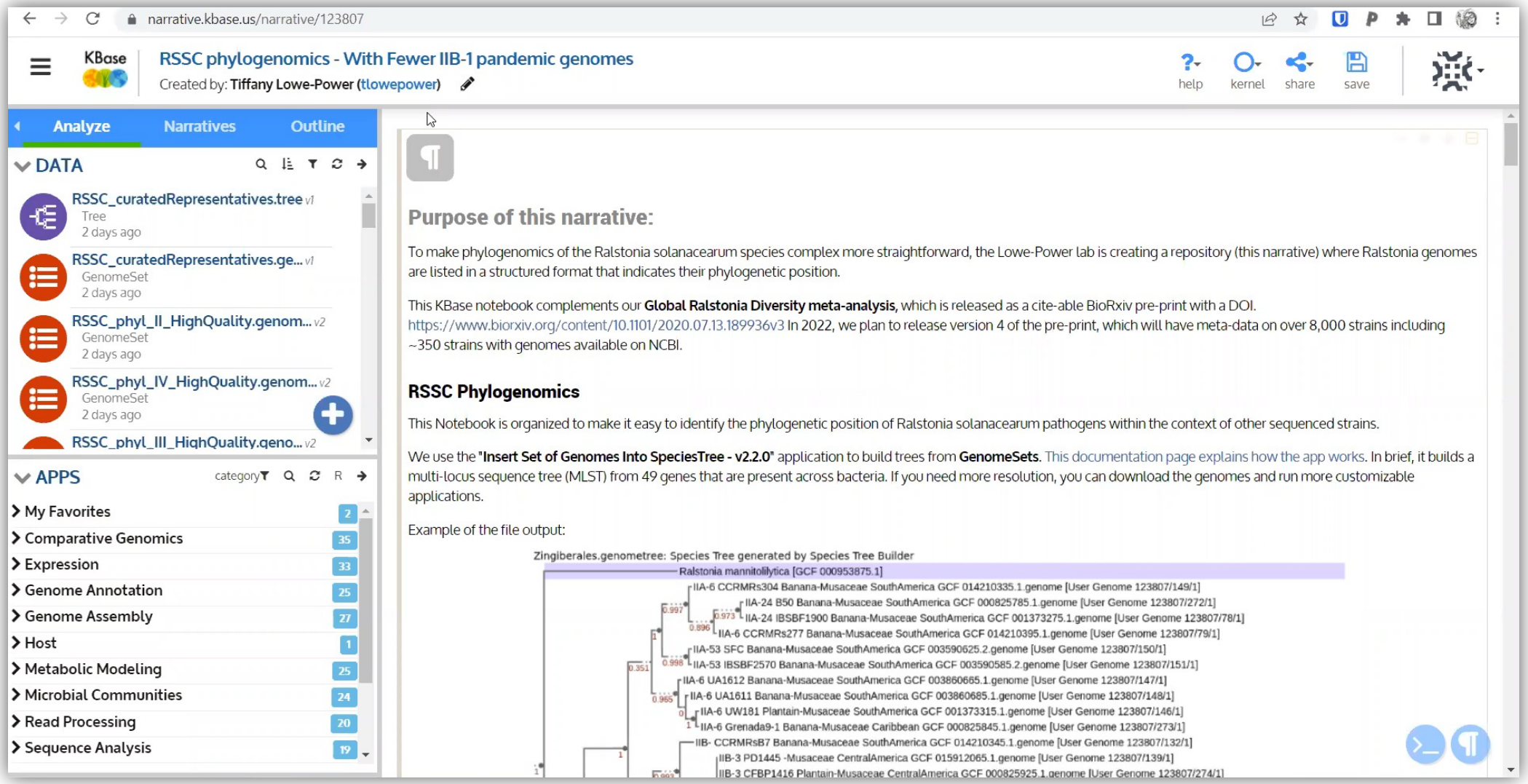
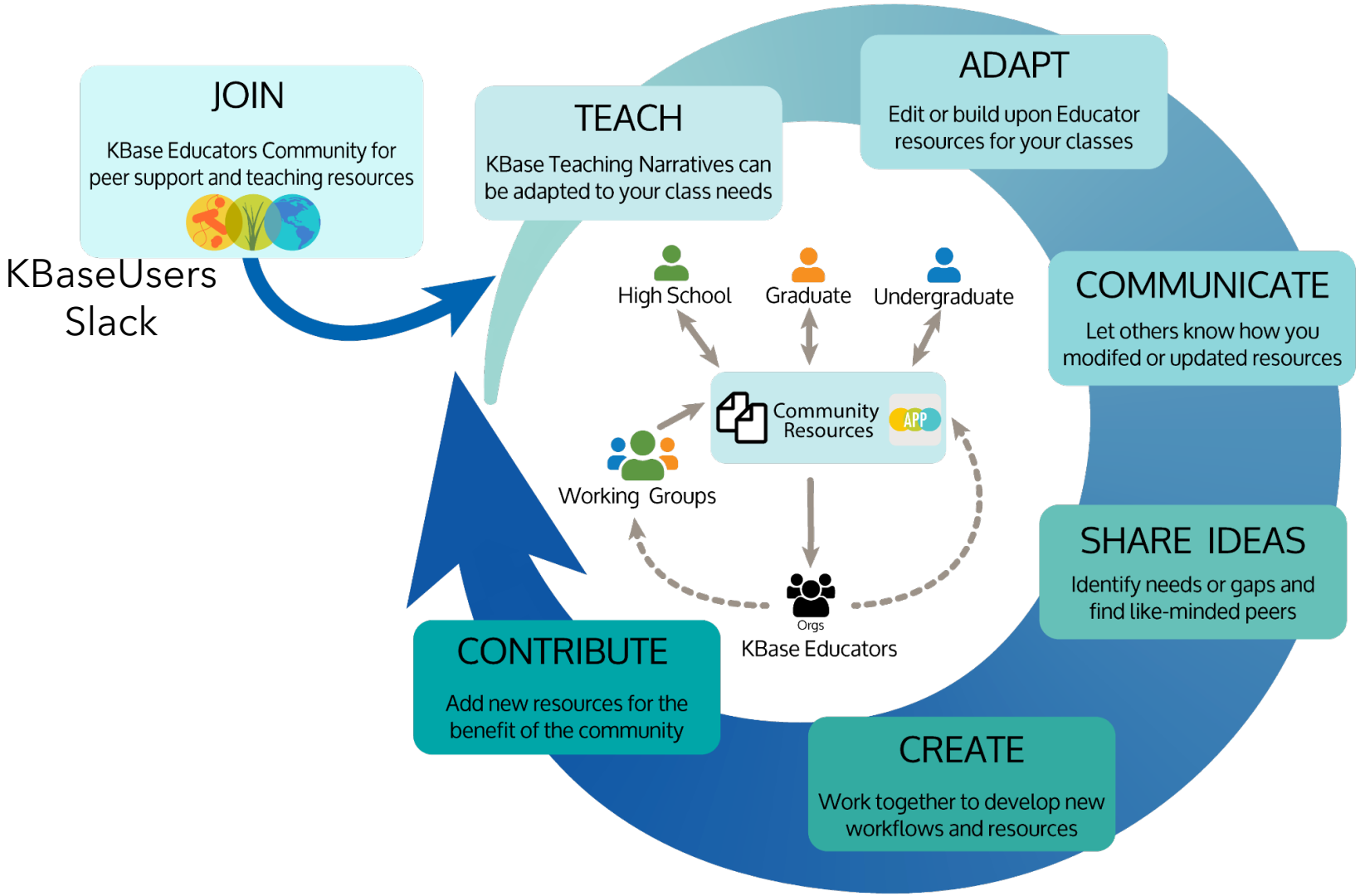
What is **KBase?**

A good platform for students to learn how to lab notebook by analyzing genomes.
No coding required, but “markdown language” to format notebook entries is a gateway to coding



There is **support** for bringing KBase into the classroom

KBase Educators community



Dr. Ellen Dow
KBase Educators Program Lead

Dr. Elisha Wood-Charlson
KBase User Working Group Lead

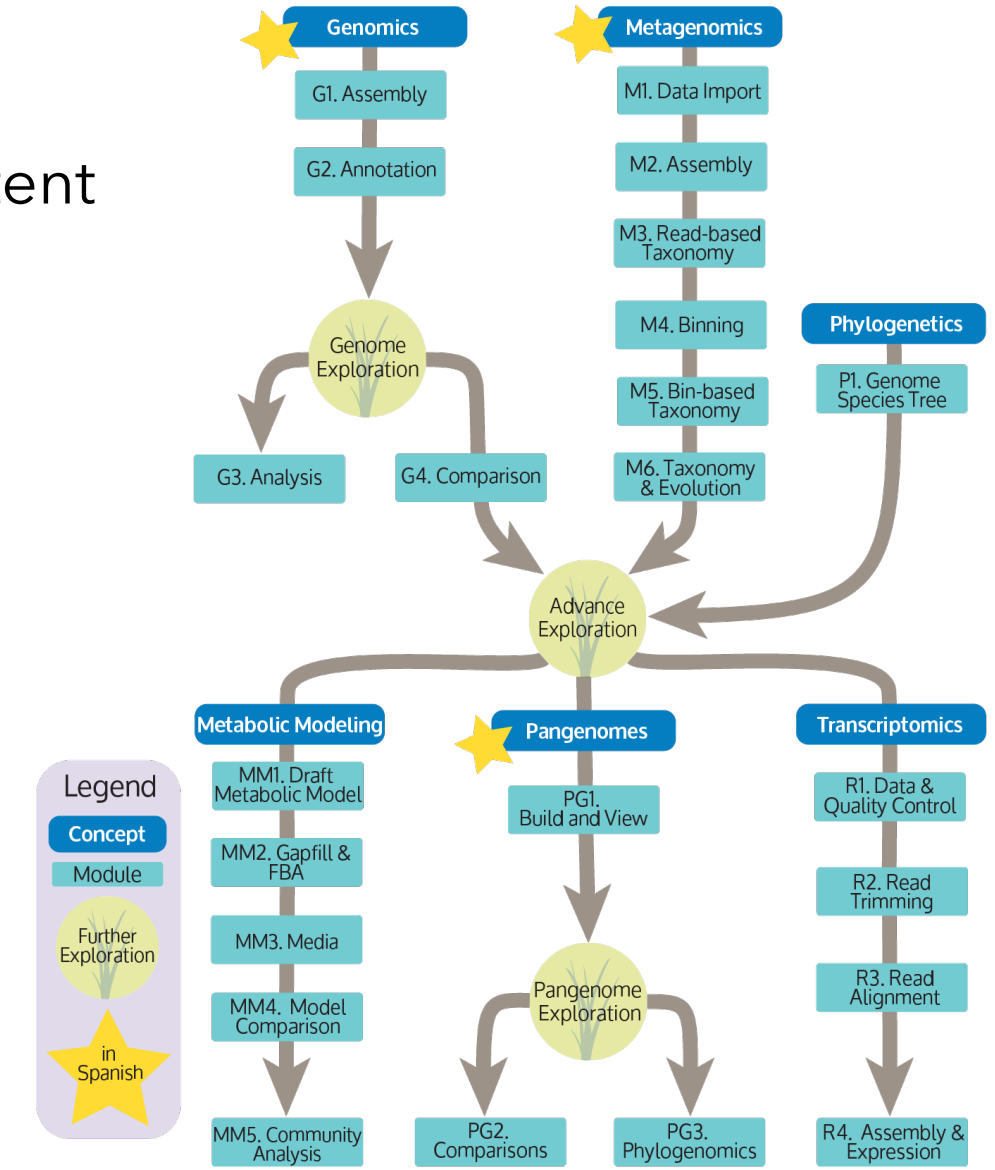
There are **resources** for bringing KBase into the classroom

Teaching modules

Adaptable – customizable, flexible content

Sharable – many existing resources!

English and Spanish



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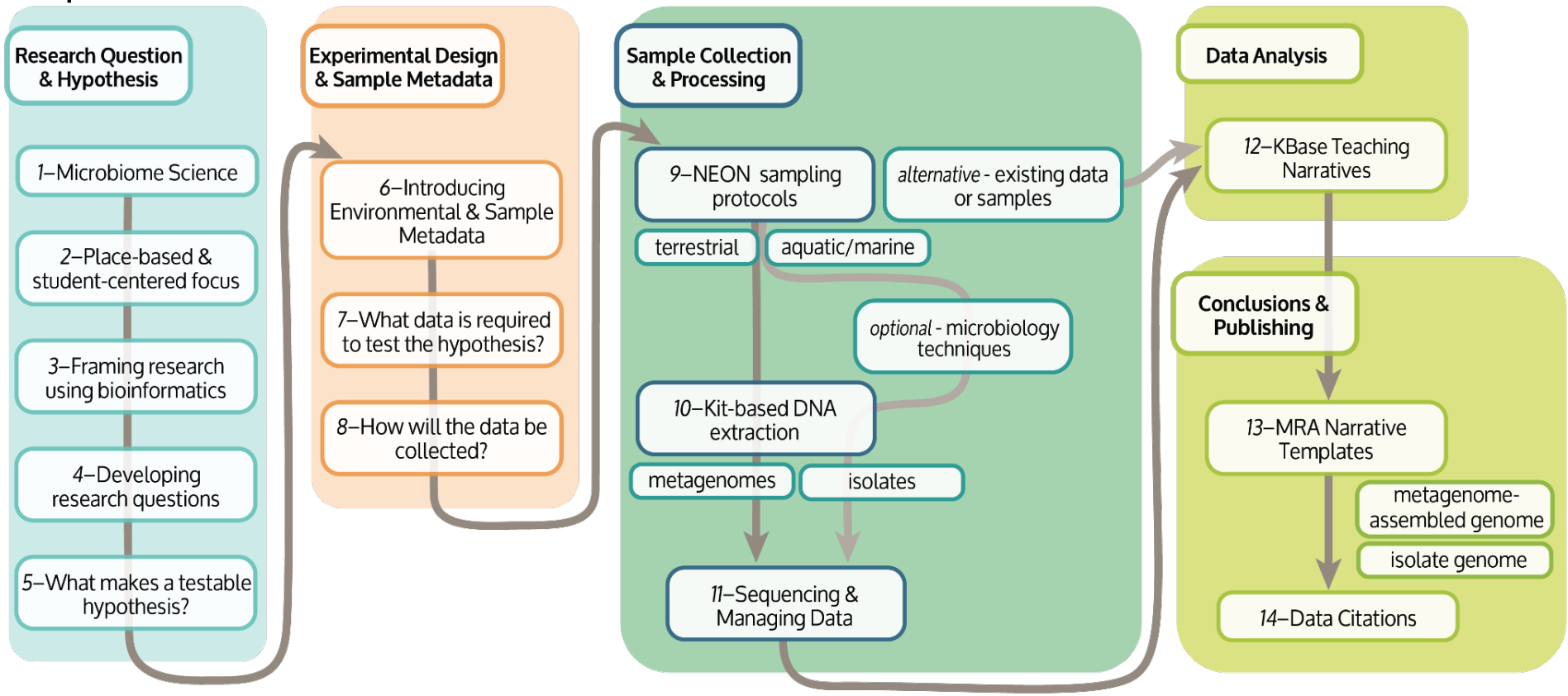
MICROnet:
Microbiomes in **C**omputational **R**esearch **O**pportunities Network

Goal: Develop modular curriculum



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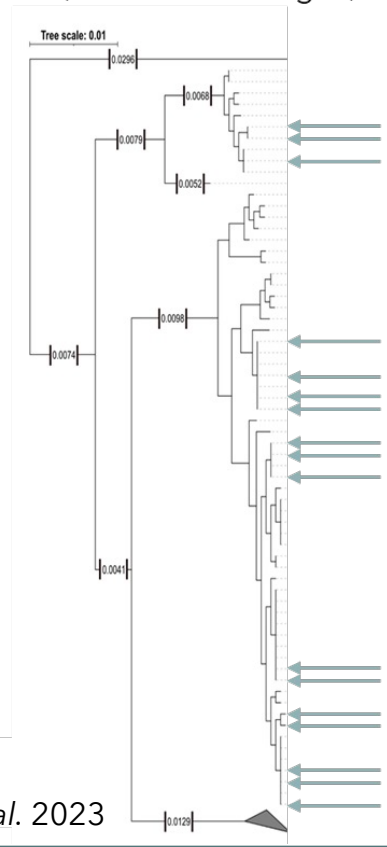
- **Contributions of *Ralstonia* genomics CURE to scientific knowledge**

Generating public *Ralstonia* genomic data

Spring 2022

17 new genomes

Collected in **Central/South America** in 1960s
by Luis Sequeira, Ivan Buddenhagan, and Ed French

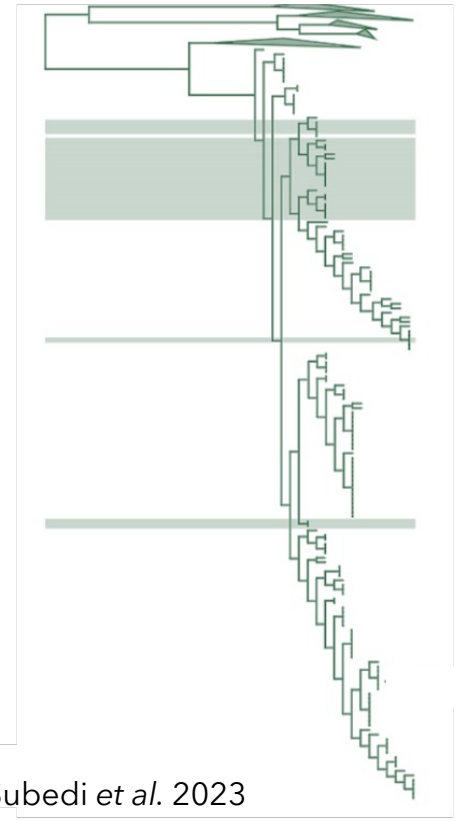


Aoun *et al.* 2023

Winter 2023

24 new genomes

Collected in **Bangladesh/Nepal** in 2019
by Nagendra Subedi and Sally Miller (Ohio State)

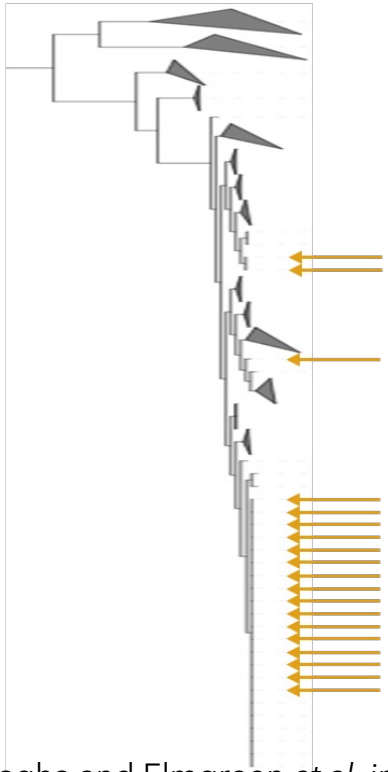


Subedi *et al.* 2023

Winter 2024

19 new genomes

Collected in **Tanzania** in 2019
by Helen Kanyagha and Sally Miller (Ohio State)



Kanyagha and Elmgreen *et al. in prep*

Release genomes to
NCBI Assembly/SRA

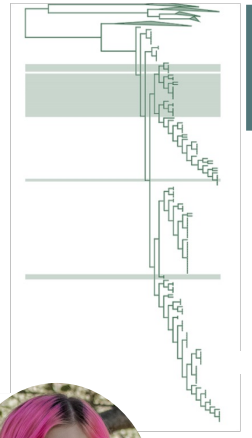
Ralstonia genomics CURE – A playground for scientific discovery

Tabitha Cowell:
Undergrad from CURE 2023

Ralstonia genomics CURE – A playground for scientific discovery

Tabitha Cowell's exploration:

Genomes of
Bangladesh/ Nepal
Ralstonia



Pangenome Analysis

Genomes had differential abundance of genes

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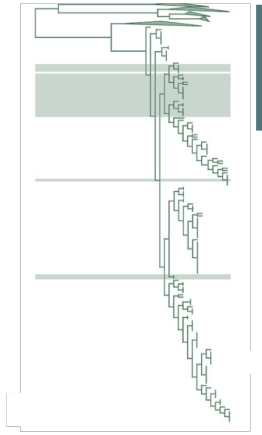

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Genomes had differential abundance of genes

"Type IV secretion TraX protein"
"Dot-Icm conjugal transfer protein"
"VirD4 conjugal coupling protein"

Tabitha noticed commonalities in gene annotations

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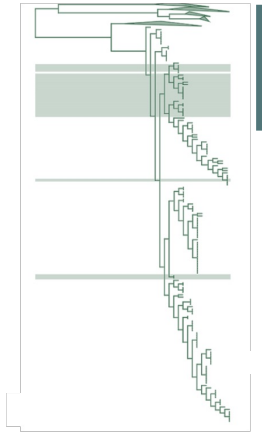

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Background knowledge

- Conjugation/T4SS relates to horizontal gene transfer
- Conjugation has not been studied in *Ralstonia*.

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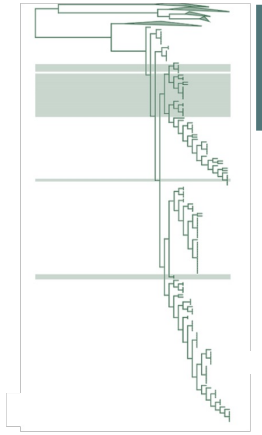

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I suggested a scientific question:

What is the phylogenetic distribution of conjugative / T4SS genes in the *Ralstonia* pangenome?

Ralstonia genomics CURE – A playground for scientific discovery

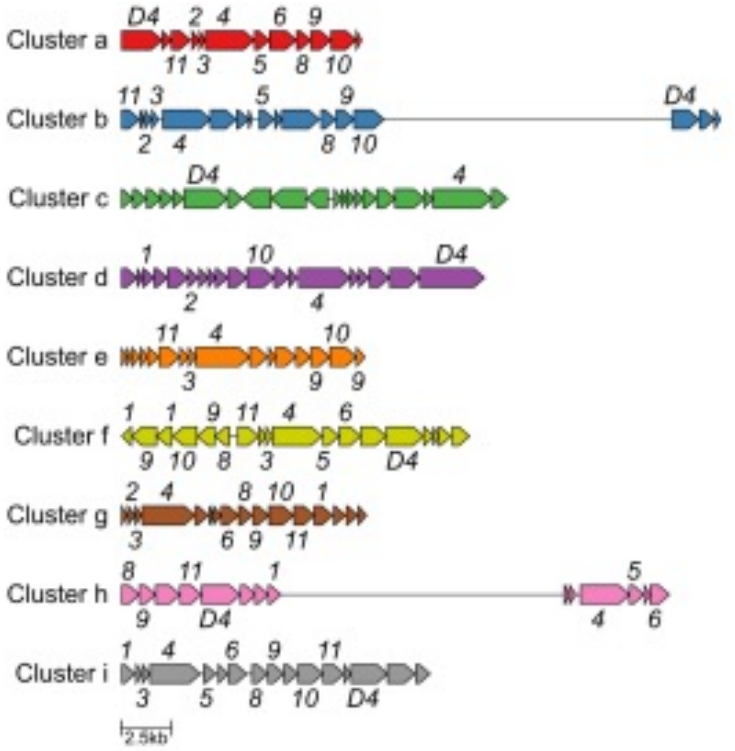
What is the phylogenetic distribution of conjugative / T4SS genes in the *Ralstonia* pangenome?

BLAST searches in KBase

Visualize phylogenetic distribution on iToL (Interactive Tree of Life)

Investigate gene organization/ synteny Clinker

Tabitha has classified 9 types of conjugative gene clusters



Ralstonia genomics CURE – A playground for scientific discovery

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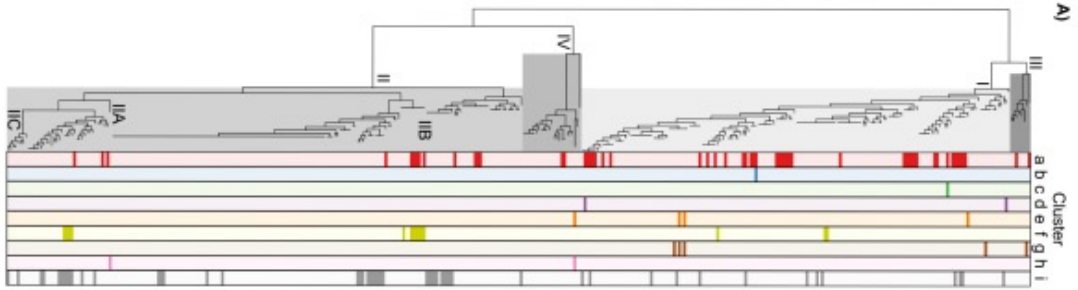
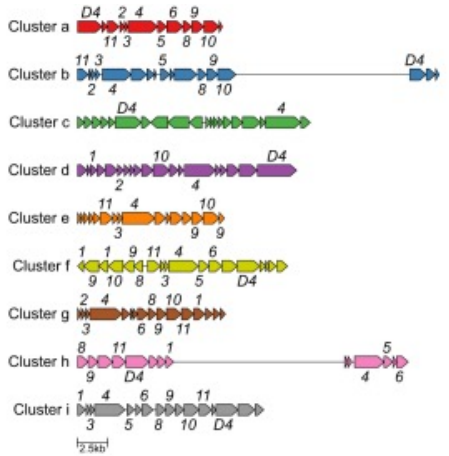
BLAST searches in KBase

Visualize phylogenetic distribution on iToL (Interactive Tree of Life)

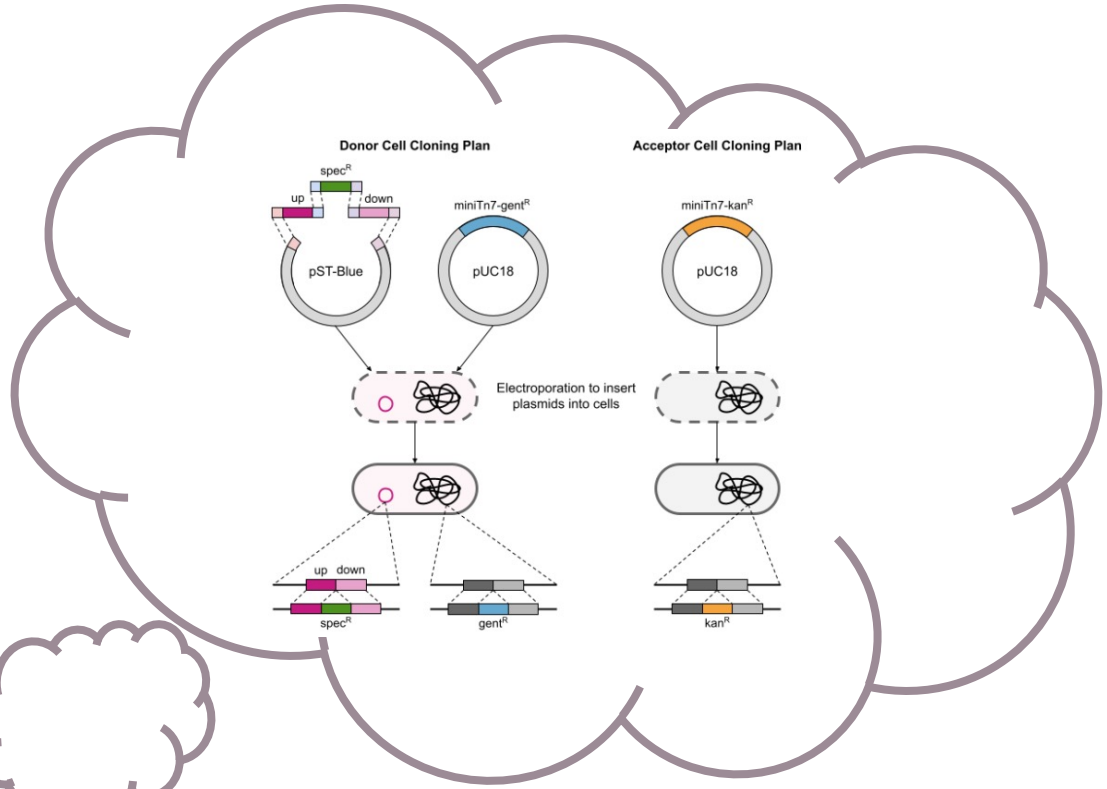
Investigate gene organization/ synteny Clinker

Tabitha discovered that conjugative/T4SS gene clusters have sporadic phylogenetic distribution, consistent with the hypothesis that these are mobile genetic elements





Ralstonia genomics CURE – A playground for scientific discovery



Closing Words

I am an **accidental plant pathologist**.

The *Ralstonia* CURE is based on my knowledge base, resources at my disposal, and the student population I work with.

How can **you** have **impact**
…on your **field?**
…on the **next generation of scientists?**

KBase
SEA PHAGES
tiny earth
Small World Initiative
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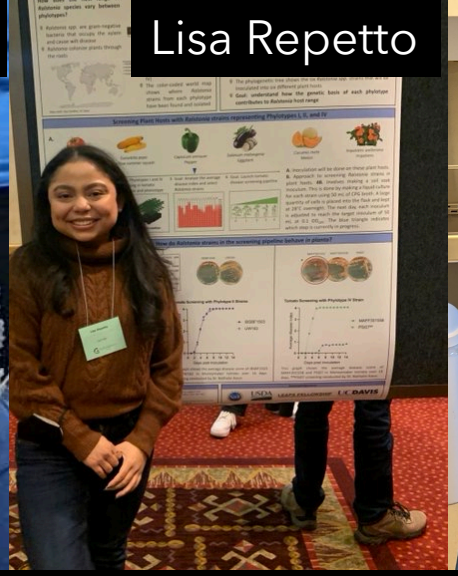
BOSIL™
iGEM


Dr. Nathalie Aoun

Jason Avalos


Lisa Repetto


Stratton Georgoulis


Vienna Elmgreen

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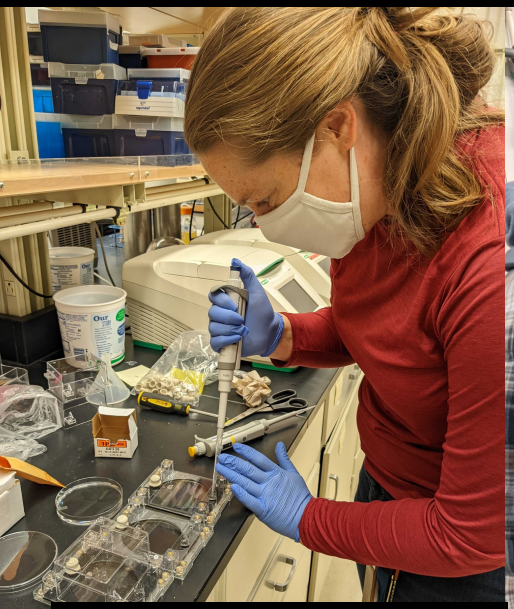
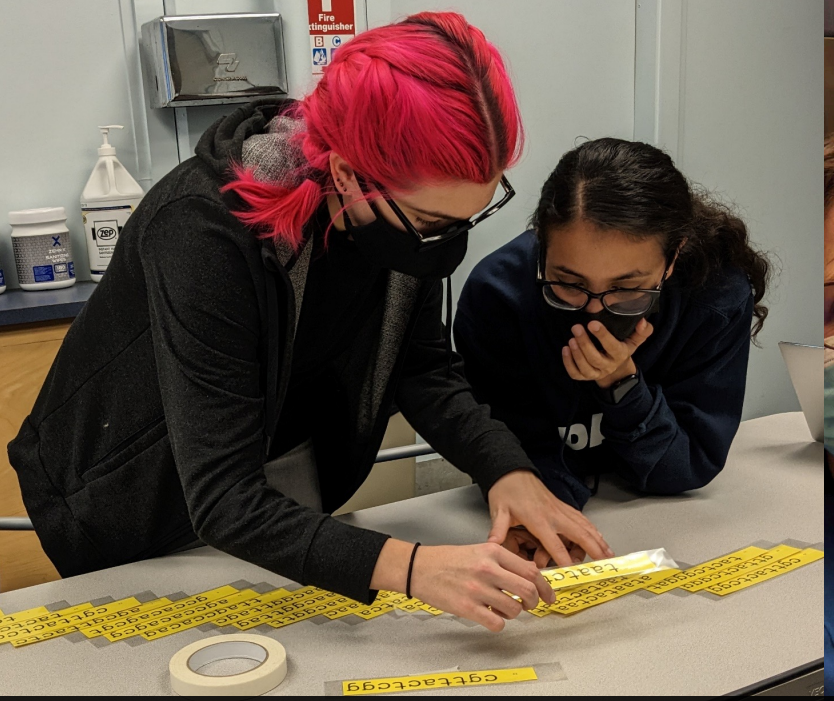
NSF USDA Hellman Fellows UCDAVIS

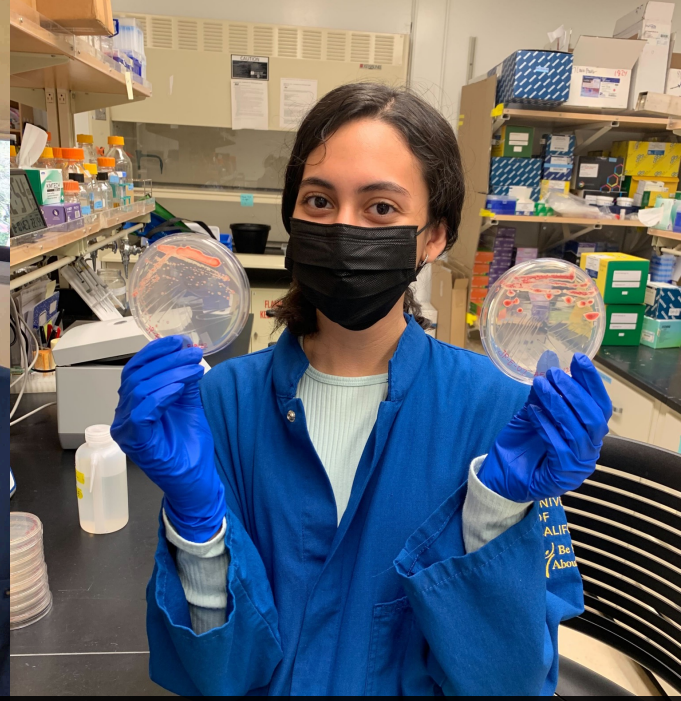

Dr. Becca Schomer


Matt Cope-Arguello


Jonathan Beutler

Agnès Bellon





Questions?