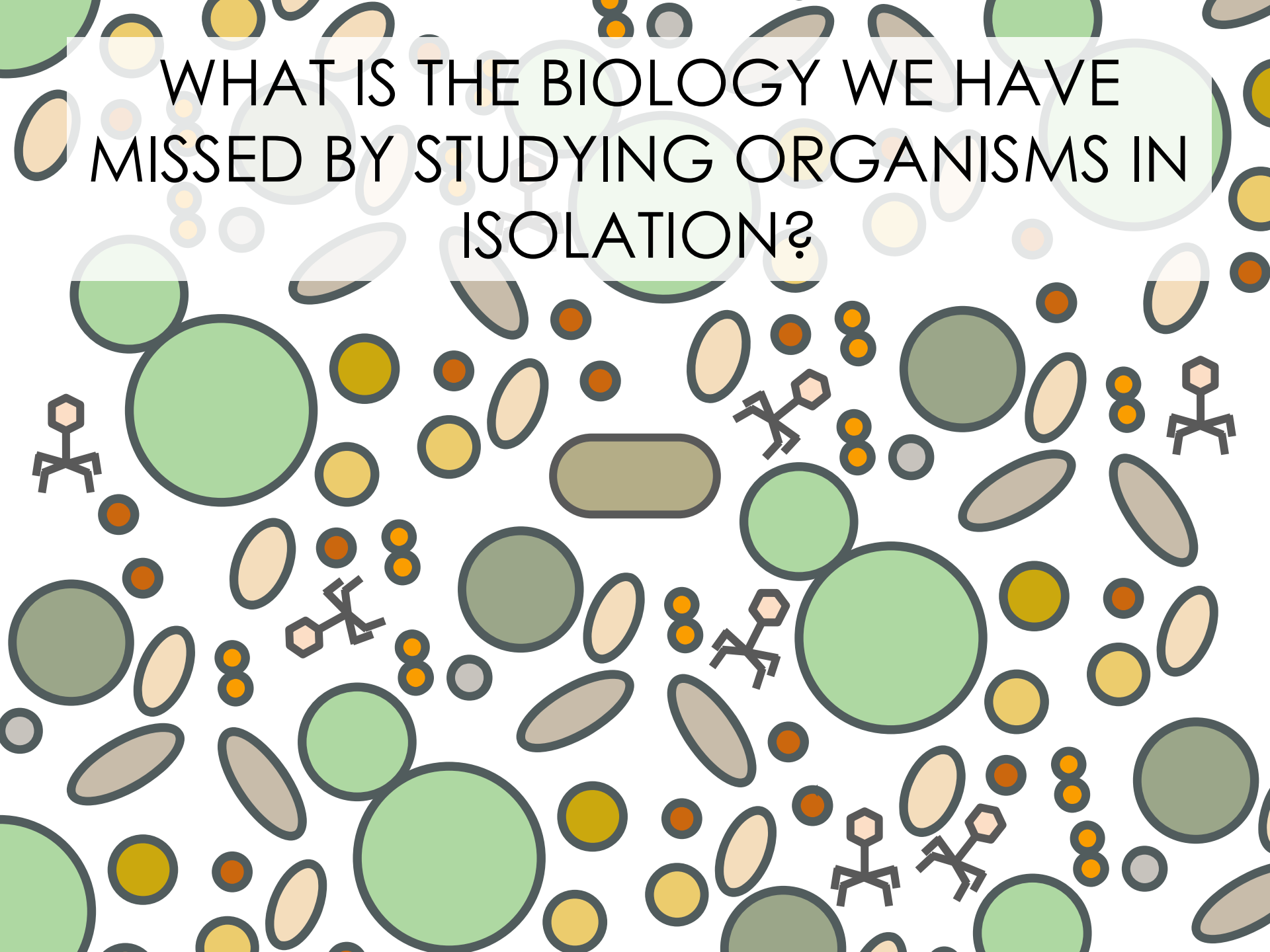




# DISSECTING SPECIES INTERACTIONS IN THE CHEESE MICROBIOME

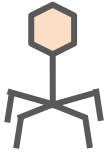
Rachel Dutton, PhD  
Associate Professor  
Division of Biological Sciences  
UC San Diego

WHAT IS THE BIOLOGY WE HAVE  
MISSED BY STUDYING ORGANISMS IN  
ISOLATION?



# MODEL SYSTEMS ARE POWERFUL

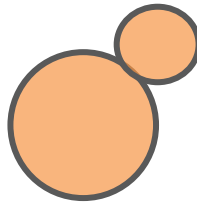
Make observations → Test hypotheses



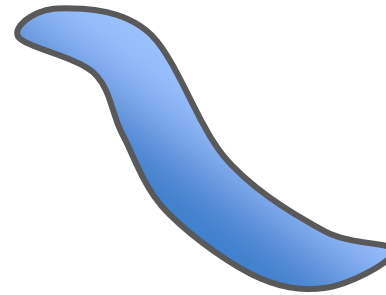
Phage  
lambda



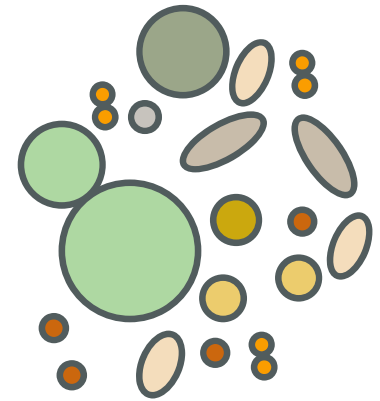
*E. coli*



*S. cerevisiae*

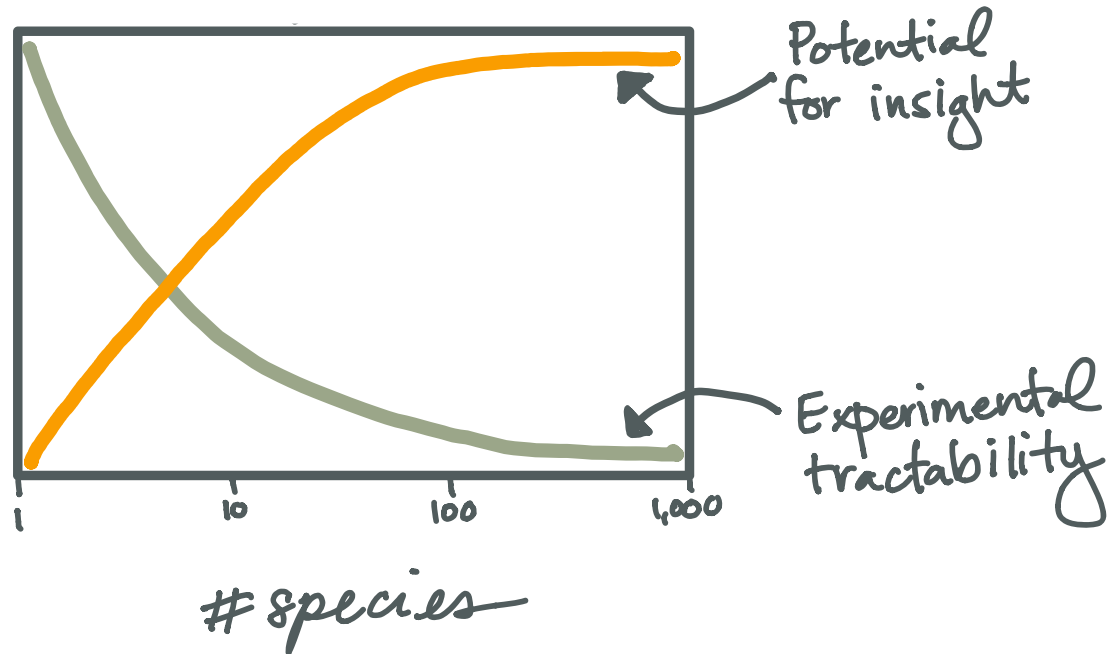


*C. elegans*

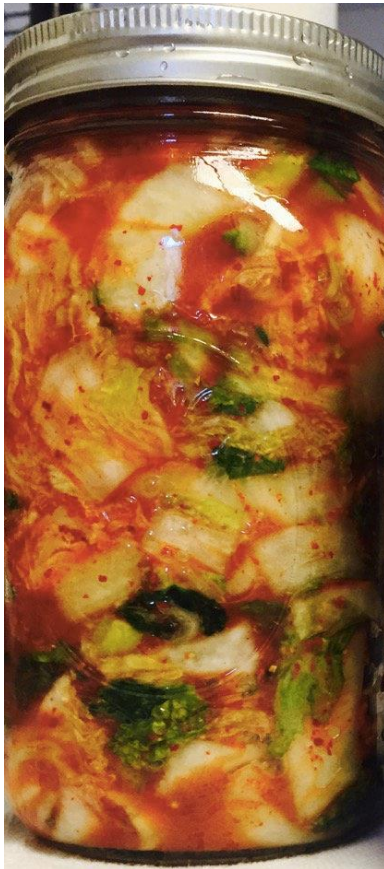


Microbiome?

# BUT WHICH COMMUNITY?



# FERMENTED FOODS FALL IN THE “GOLDBLOCKS ZONE”



kimchi



bread



kombucha



beer



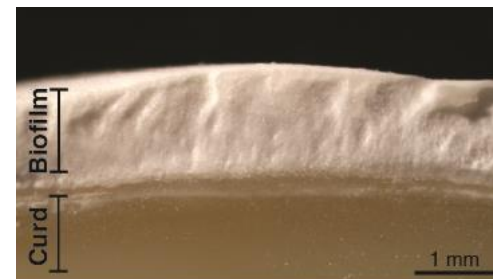
cheese

# THE CHEESE RIND BIOFILM

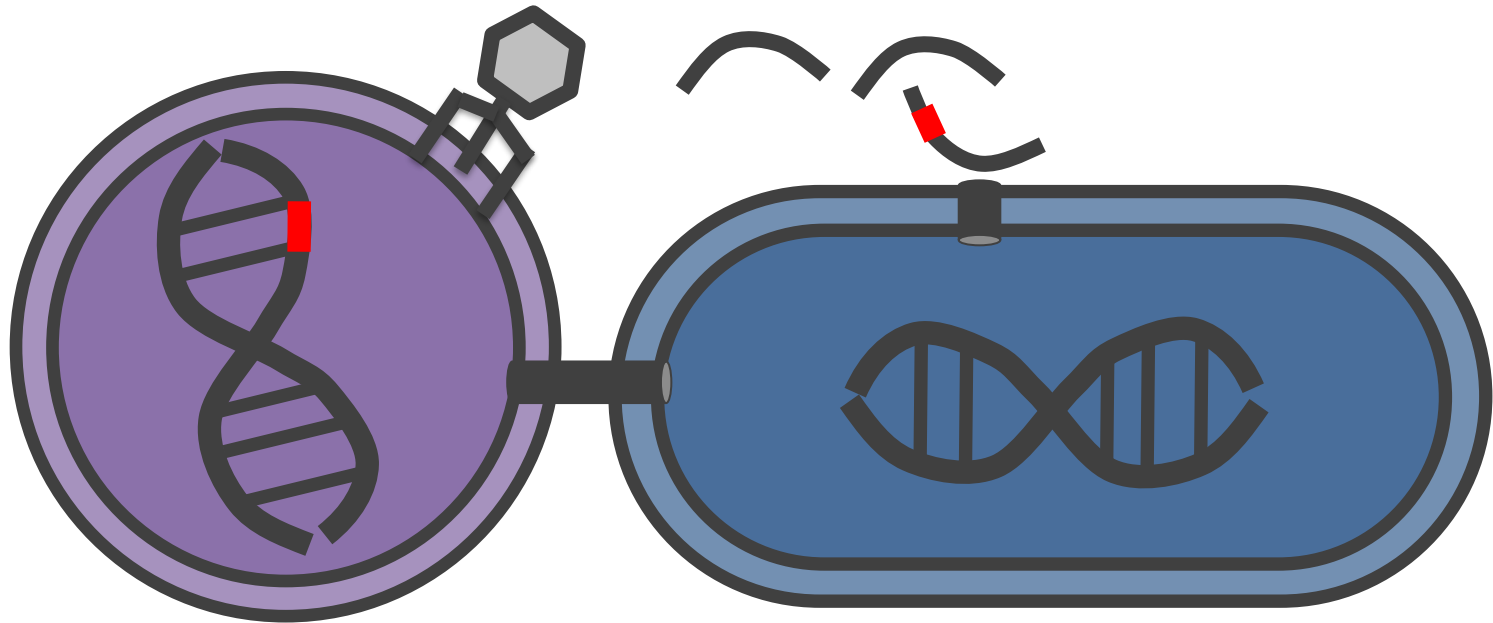
Communities range from low to medium complexity, phylogenetically diverse, completely culturable, amenable to *in vitro* reconstruction

*in situ*

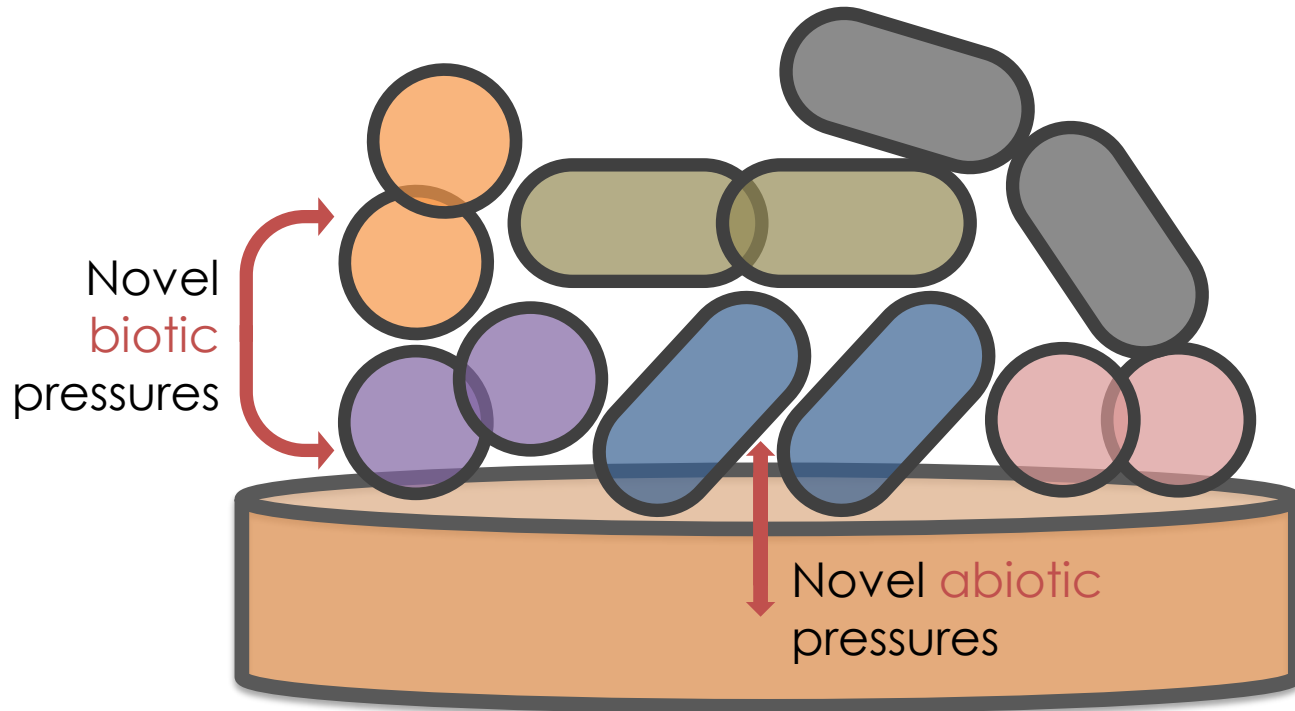
*in vitro*



# HORIZONTAL GENE TRANSFER OCCURS WITHIN COMMUNITIES



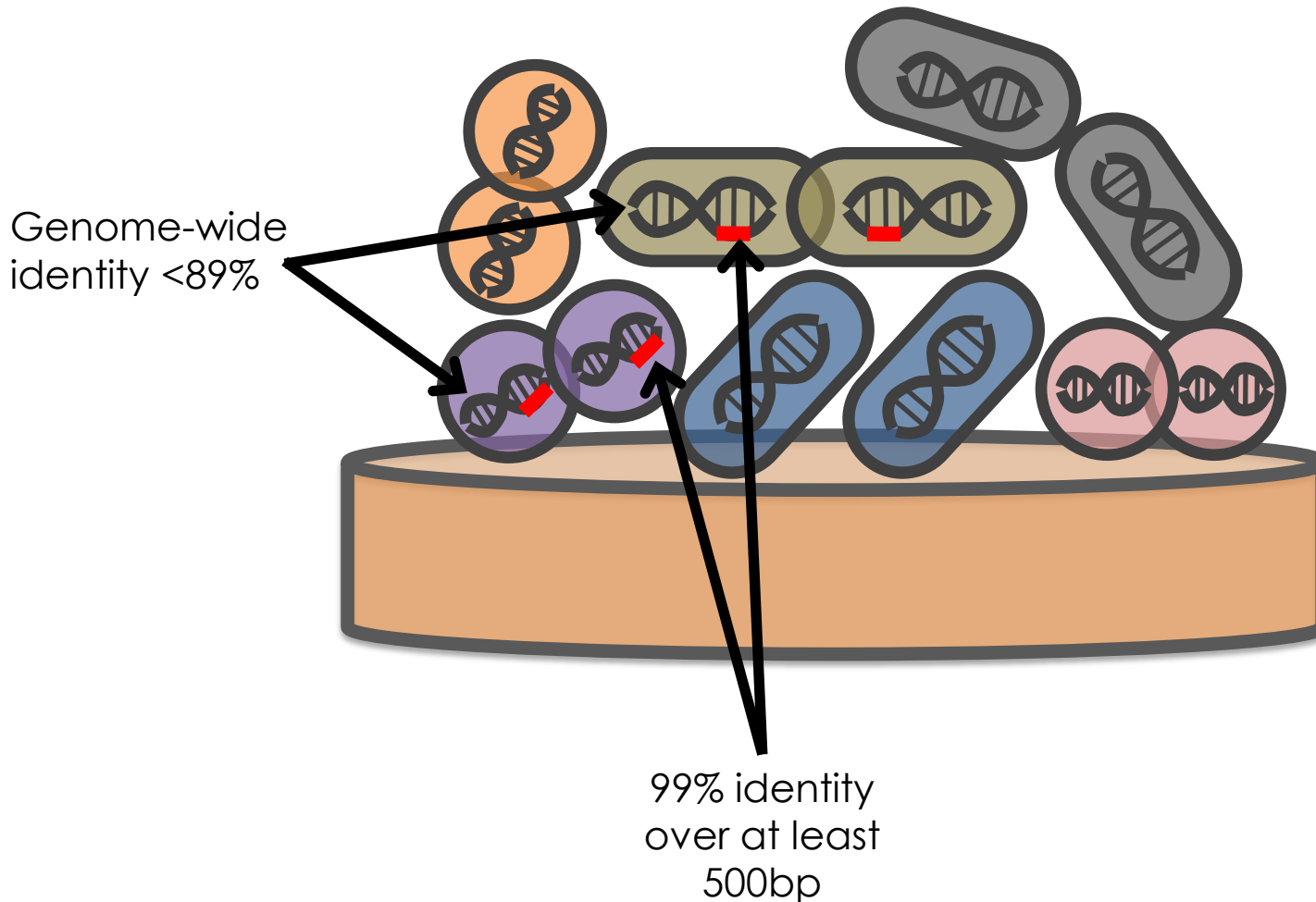
# CAN WE USE CHEESE RIND MICROBIOMES TO STUDY HGT?





# WHAT GENES ARE HORIZONTALLY TRANSFERRED?

Looked for regions of high identity between pairs of genomes (Smillie *et al*, 2011)

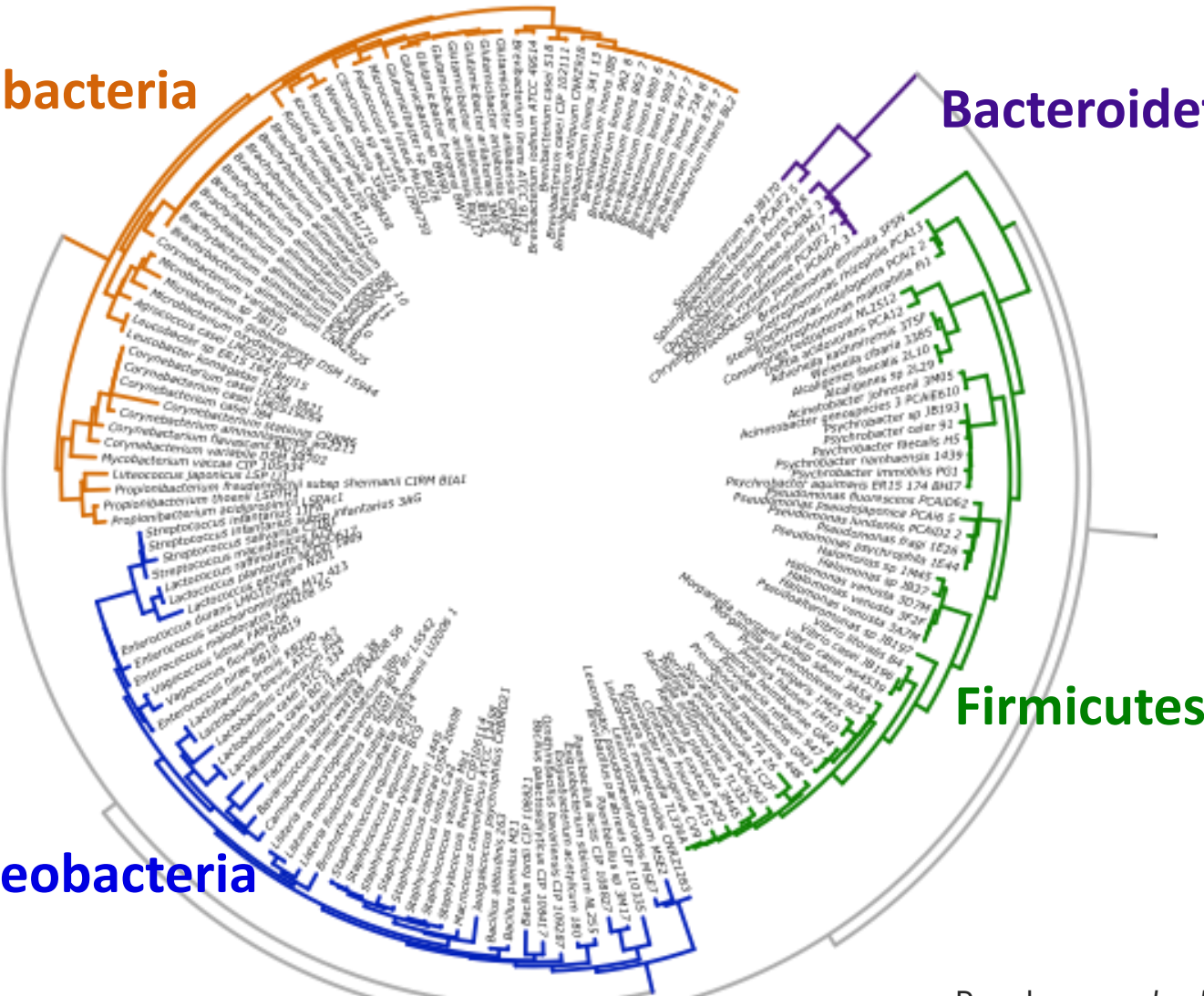


Kevin Bonham

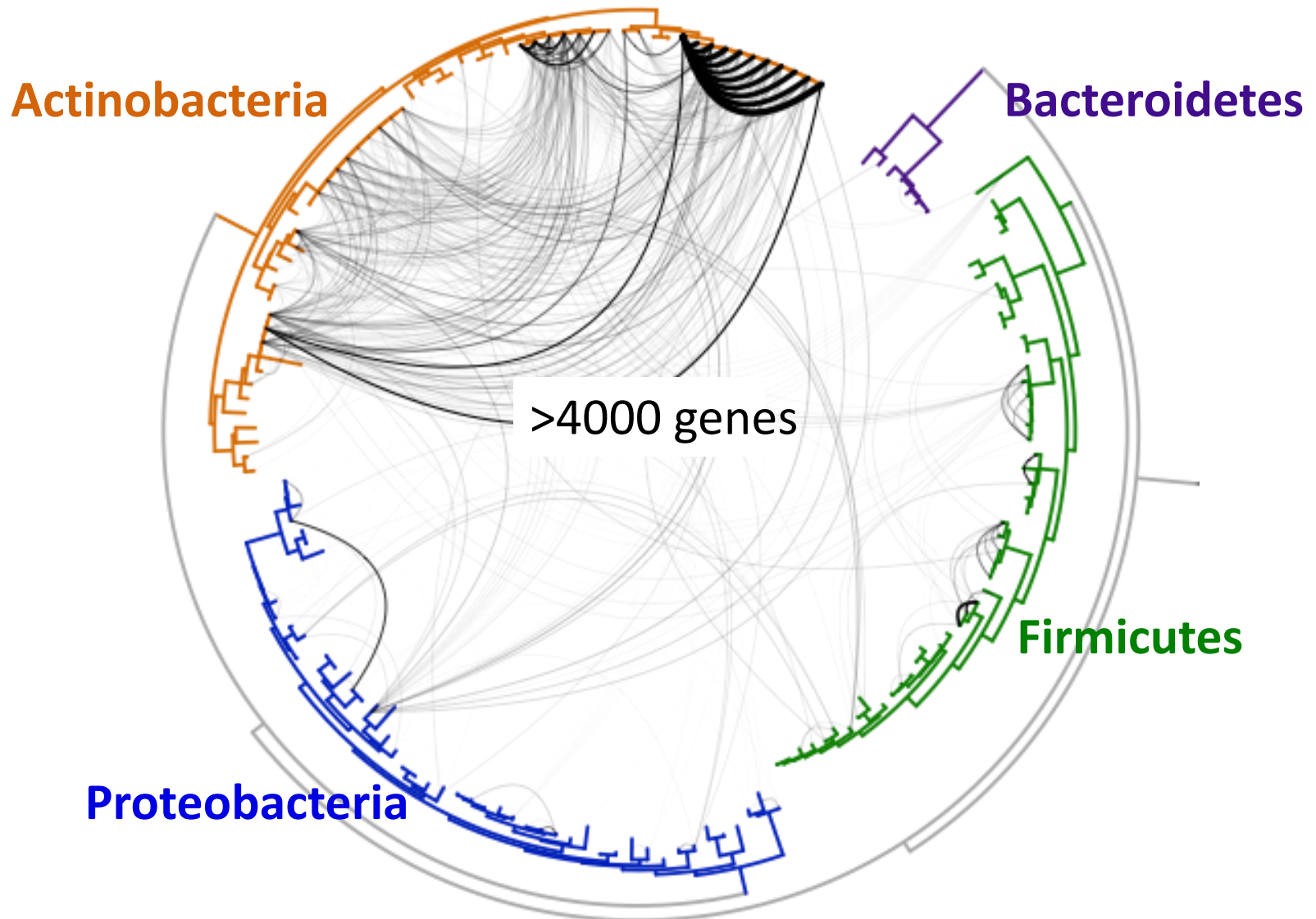
# 165 CHEESE-ASSOCIATED BACTERIAL GENOMES

Actinobacteria

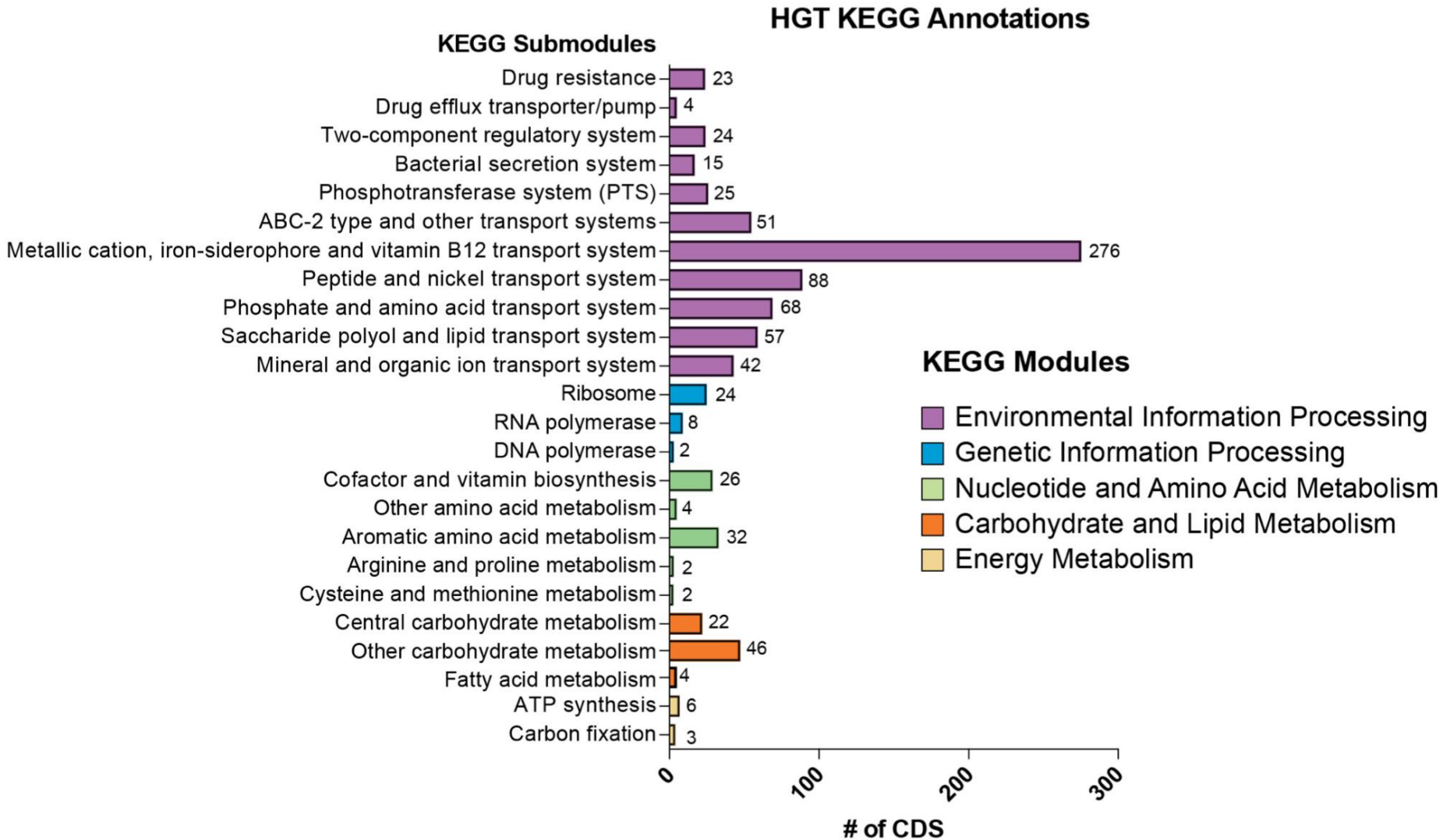
Bacteroidetes



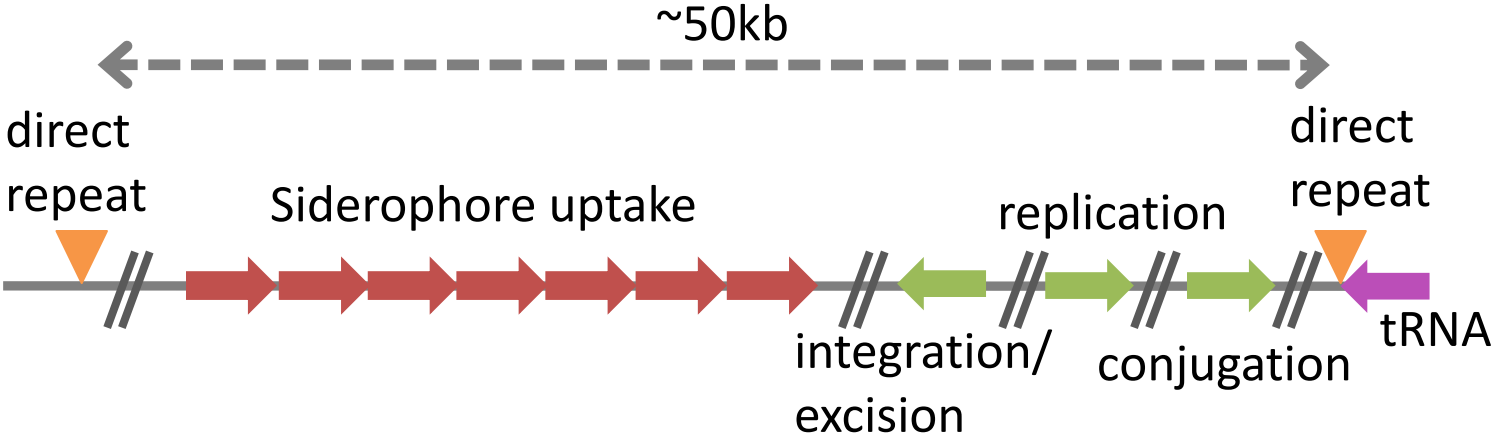
# EXTENSIVE HGT IN CHEESE



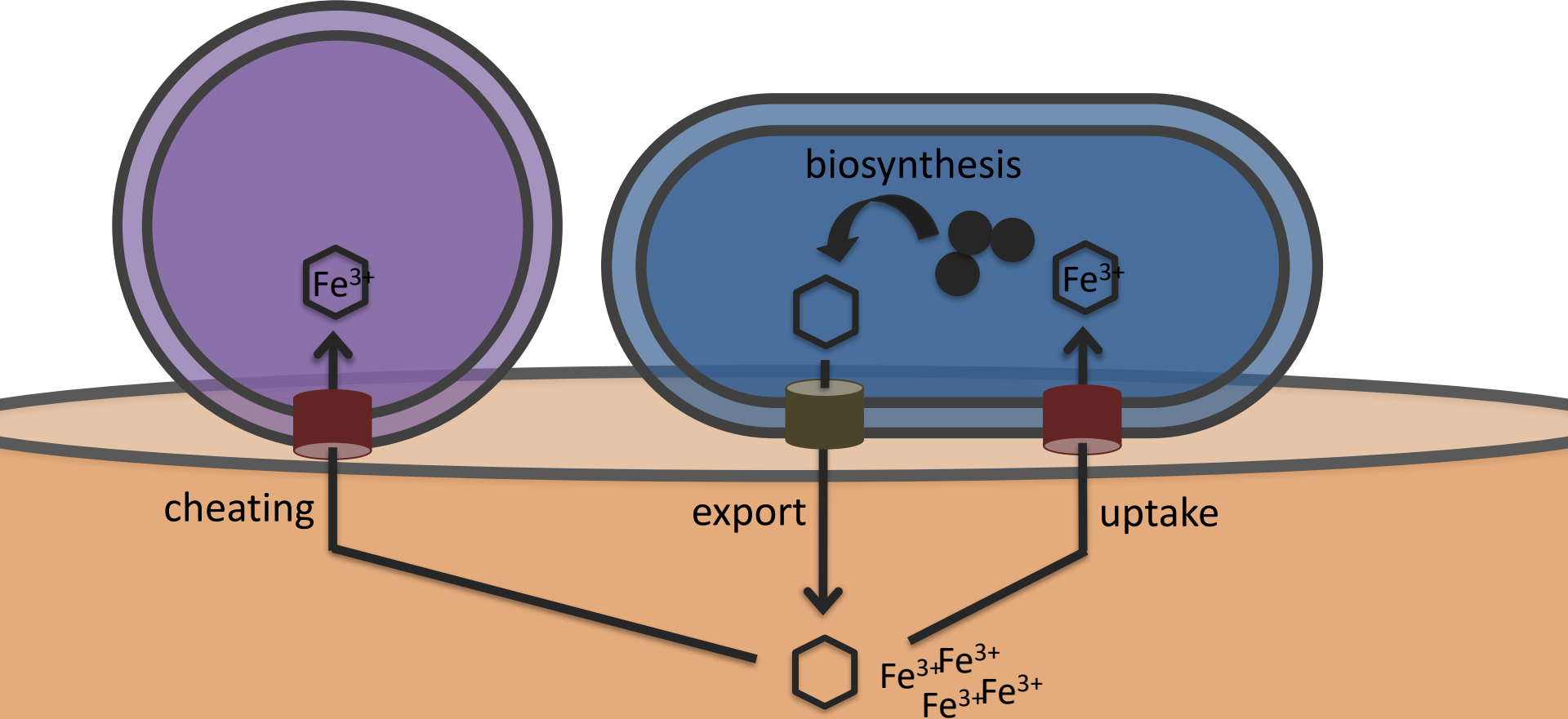
# TRANSPORT SYSTEMS, ESPECIALLY FOR IRON, FOUND IN HGT REGIONS



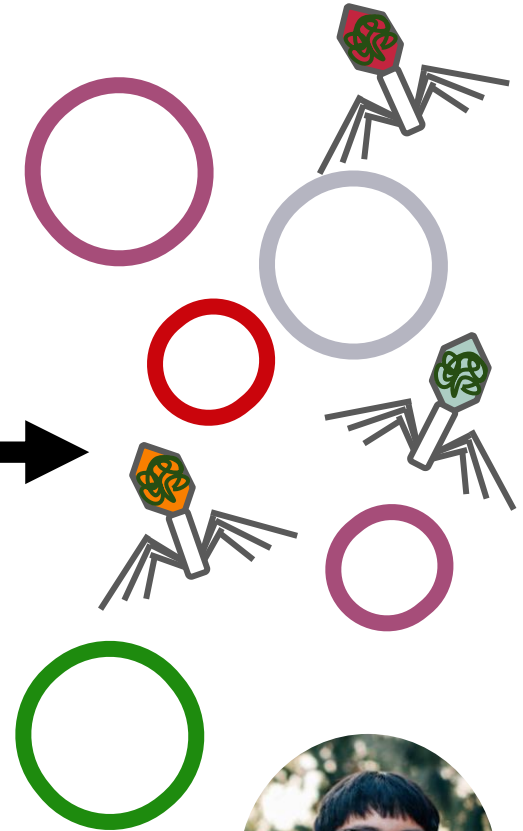
# SIDEROPHORE UPTAKE GENE CLUSTERS REPEATEDLY FOUND ON MOBILE ELEMENTS



# POTENTIAL CHEATING IN CHEESE MICROBIOMES



# WHAT IS THE DIVERSITY OF PLASMIDS AND PHAGE IN CHEESE RINDS (AND KEFIR)?



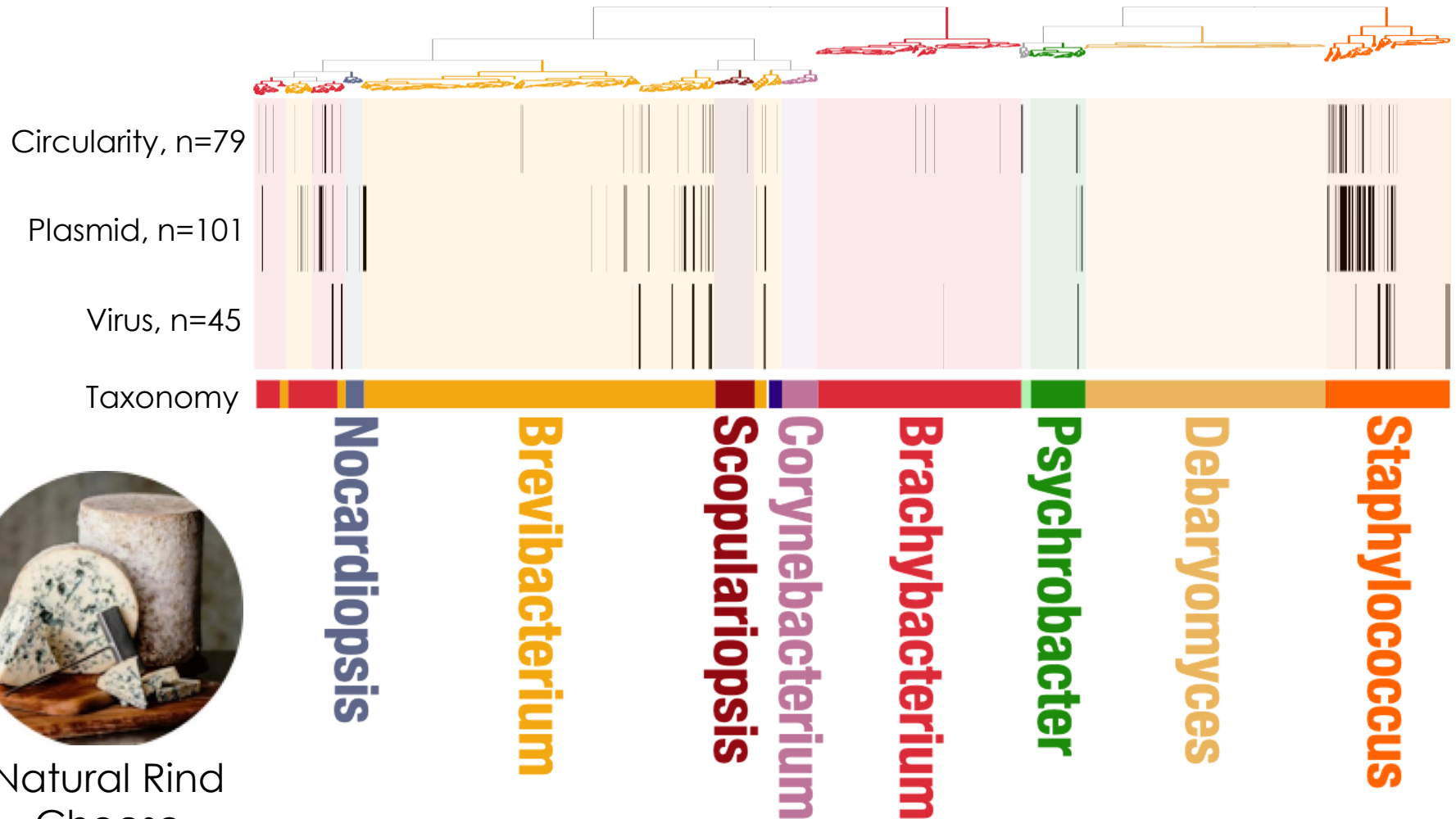
Sequencing: minION 9.4  
Basecalling: guppy 4.0.15  
Assembly: metaFlye  
Polishing: racon, medaka, DIAMOND/MEGAN  
plasmids: plasflow, PlasmidVerify, plasmidDB  
viruses: DeepVirFinder, VirSorter, ViPTree, vcontact2  
Mobilization: mob-suite  
Visualization: anvi'o, bandage



Cong Dinh  
MD/PhD student

# LONG-READ METAGENOMICS REVEALS ABUNDANT PLASMIDS AND VIRUSES

Clustering of contigs based on tetramer frequency and contig coverage (Anvi'o)

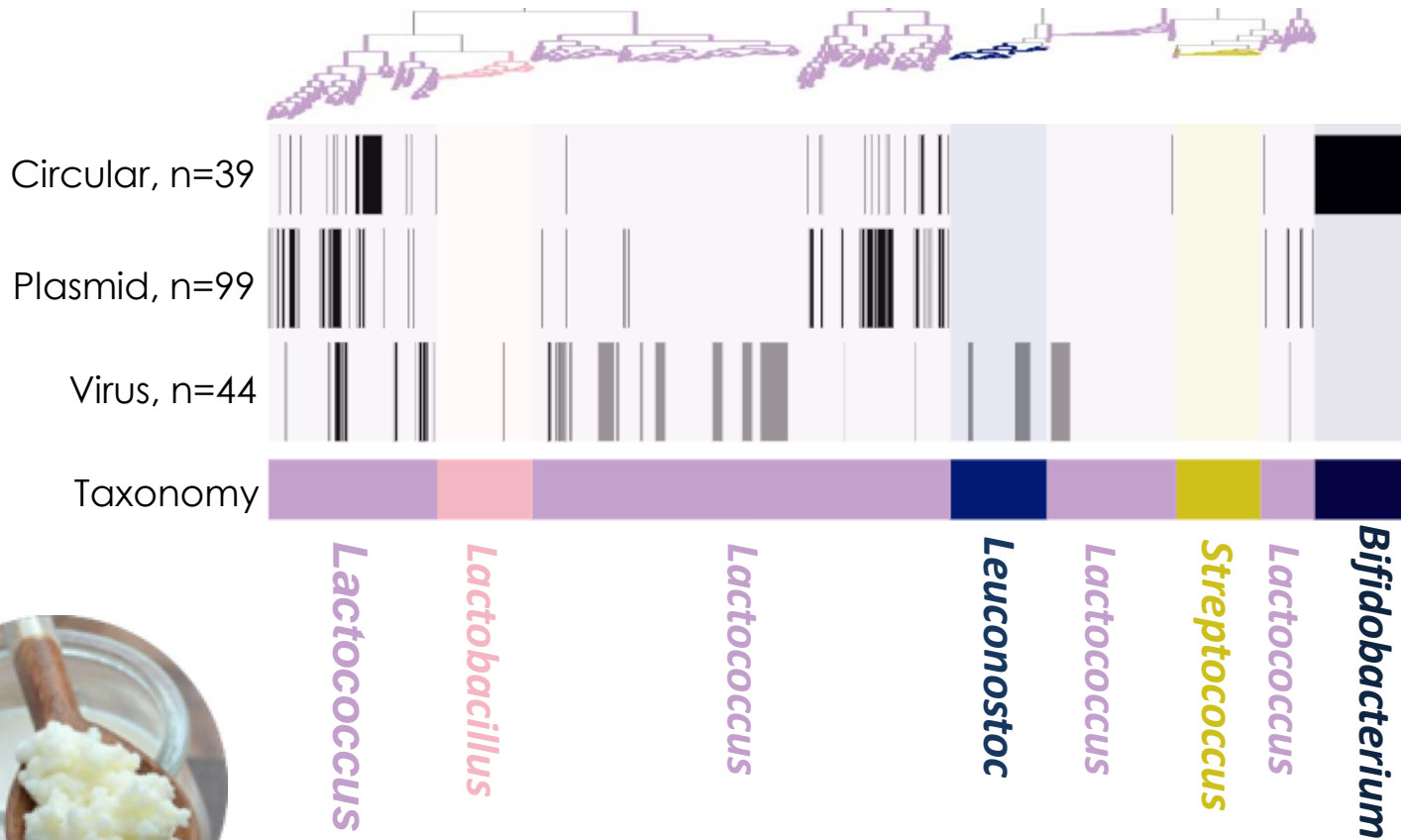


Natural Rind  
Cheese



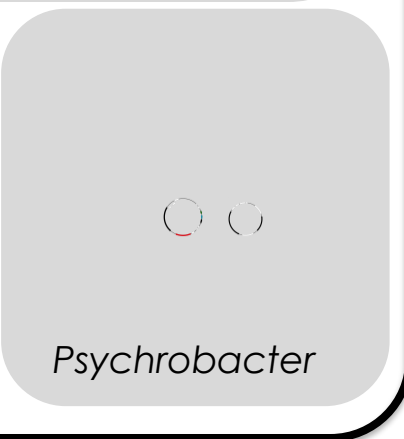
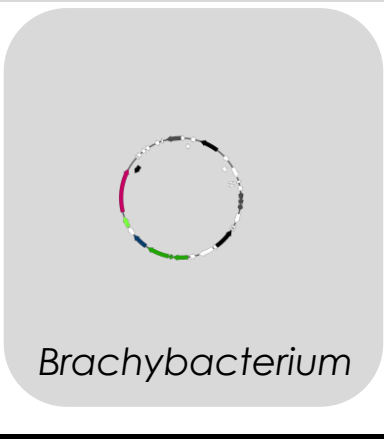
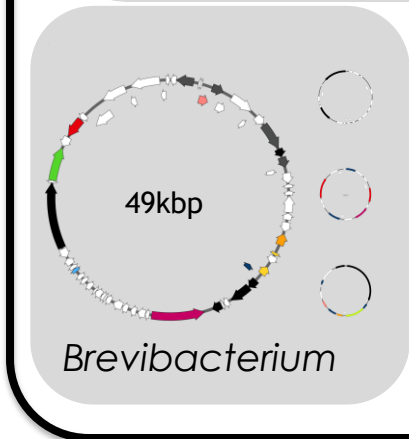
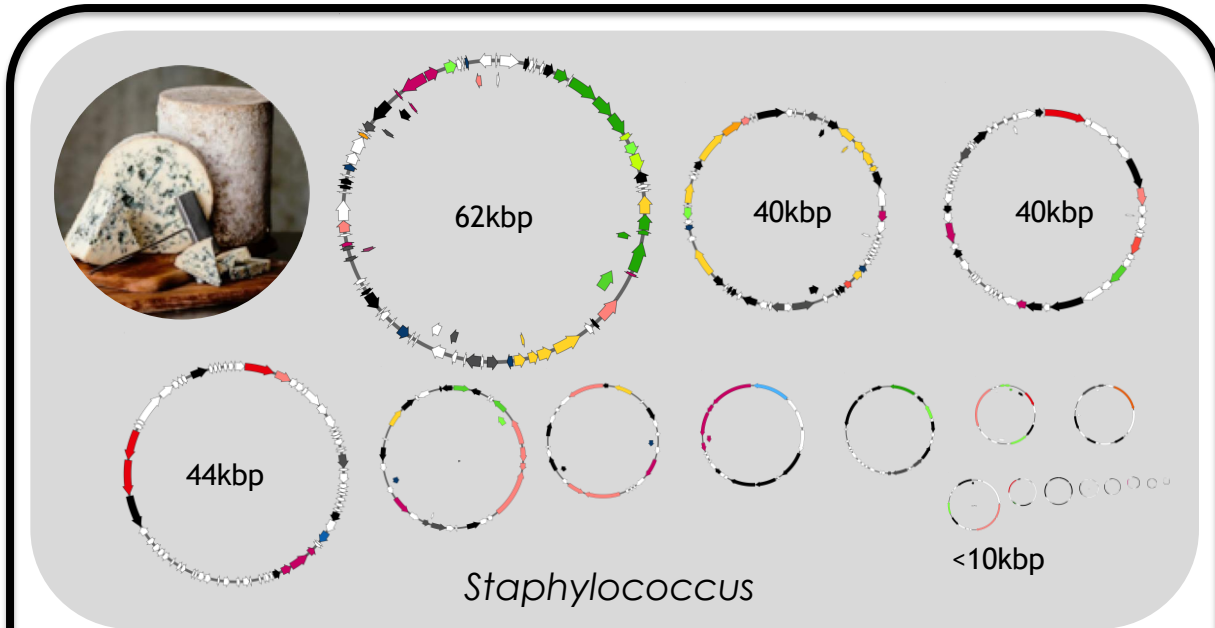
# LONG-READ METAGENOMICS REVEALS ABUNDANT PLASMIDS AND VIRUSES

Clustering of contigs based on tetramer frequency and contig coverage (Anvi'o)

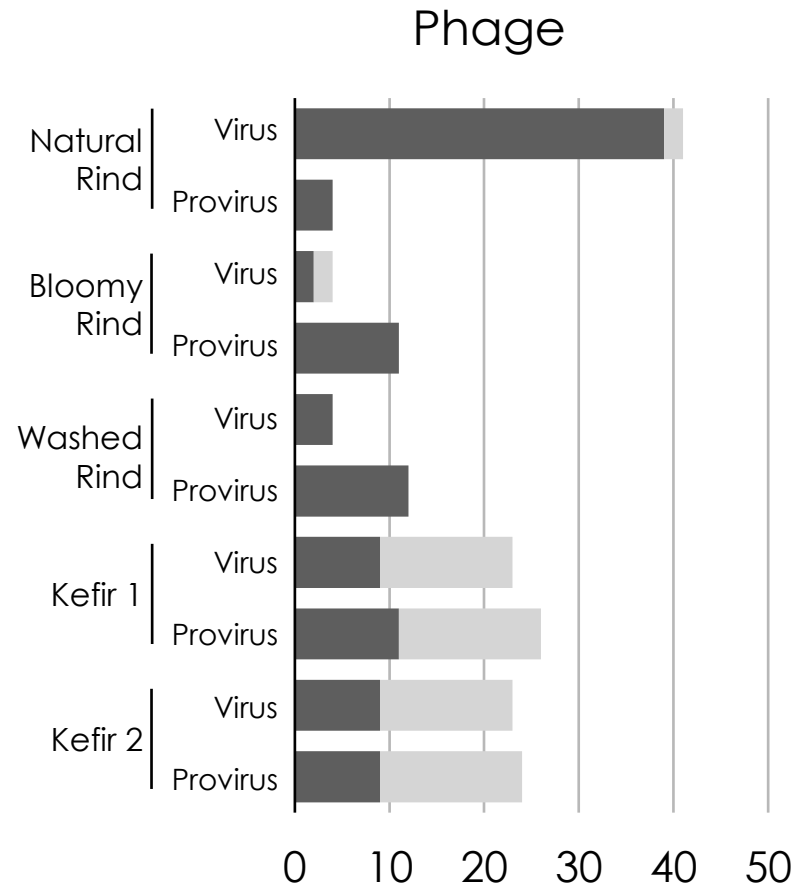
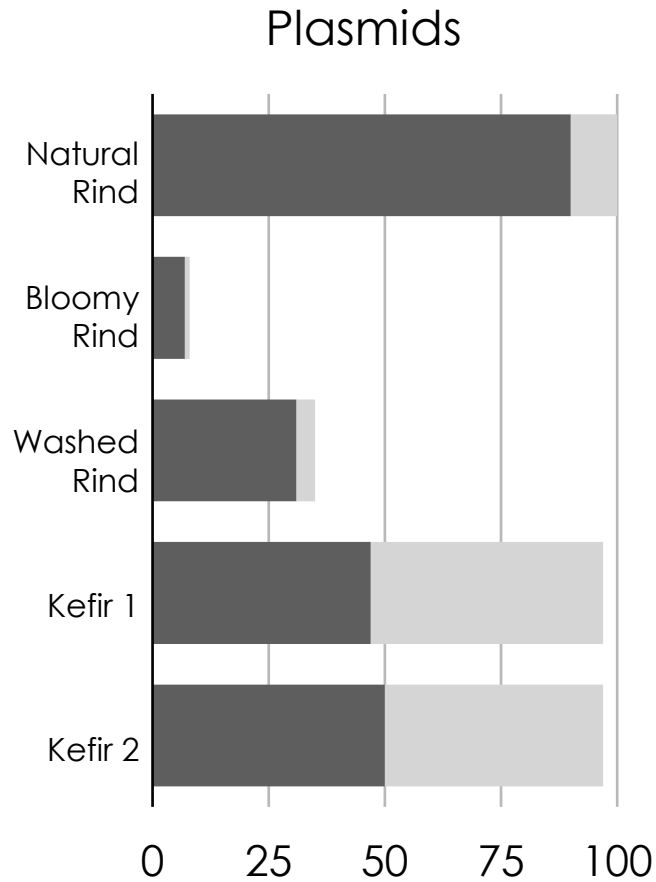


Kefir

# LONG READS ALLOWS ASSEMBLY OF CIRCULAR PLASMIDS



# MANY NOVEL PLASMIDS AND PHAGE IN CHEESE RINDS



■ Low identity to sequences in databases  
■ High identity to sequences in databases

# PLASMIDS MAY EXPAND HOSTS' CARBON ACCESS

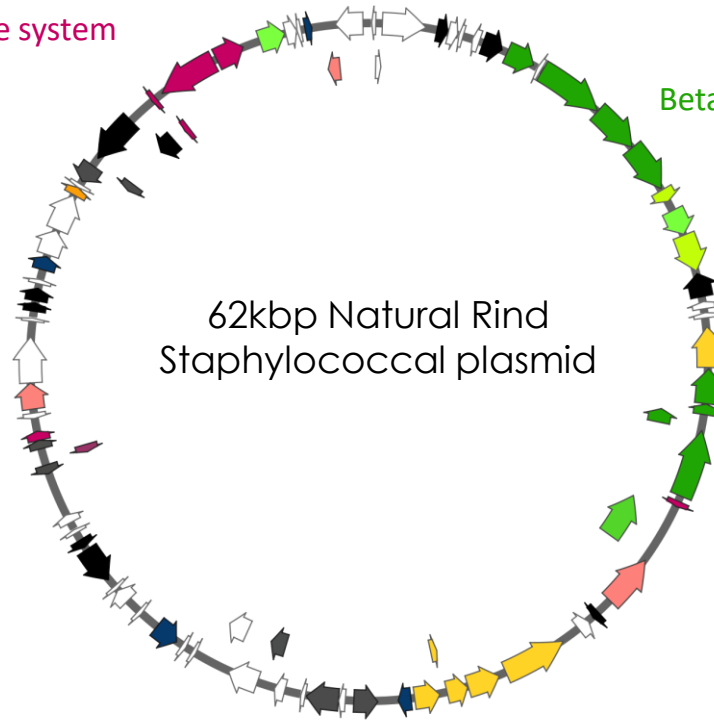
Beta-lactam resistance system

Beta-glucosidase

Carbohydrate dehydrogenases

62kbp Natural Rind  
Staphylococcal plasmid

Replication (RepA)

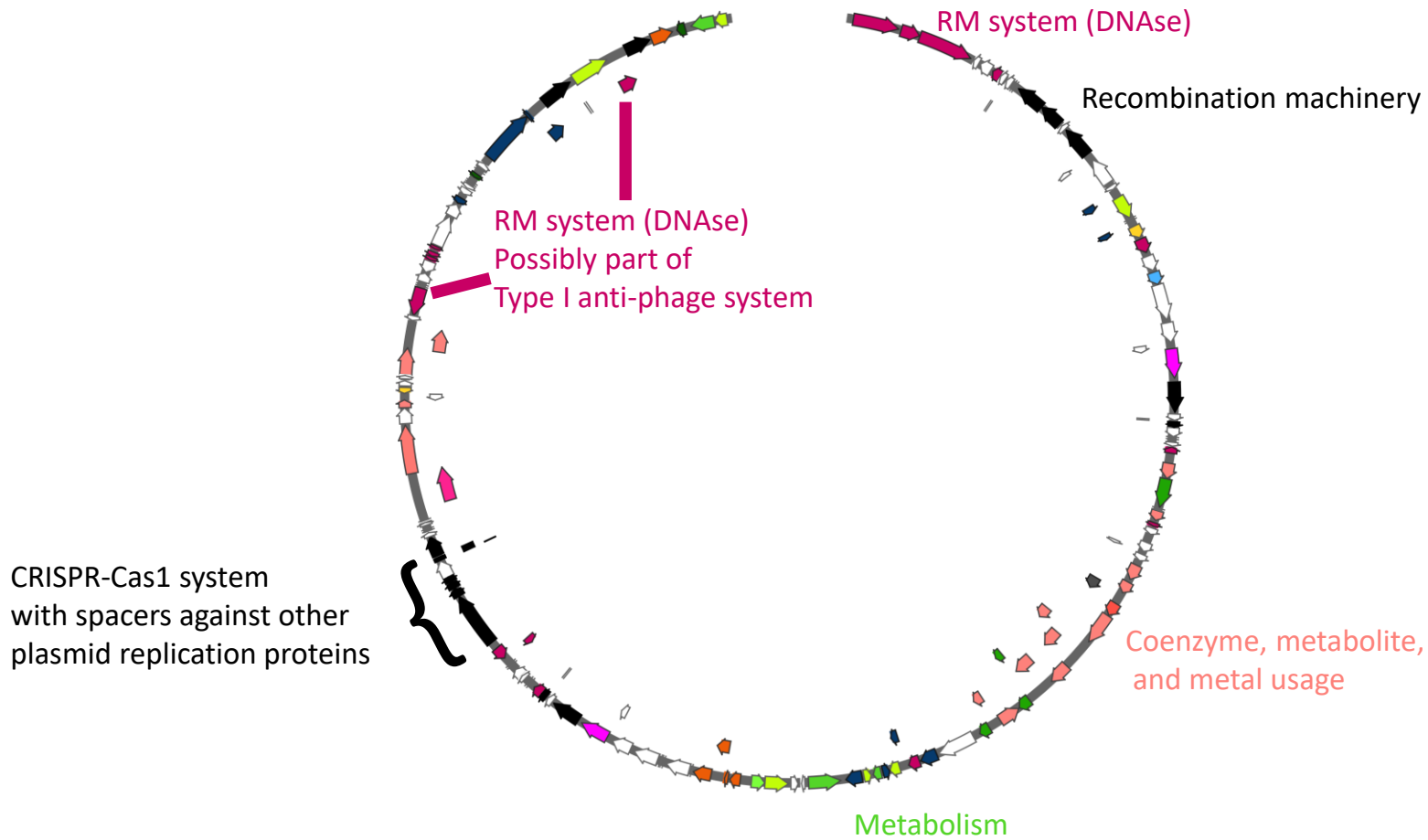


# METAL RESISTANCE AND UPTAKE IS COMMON

especially **cadmium-resistance**



# DEFENSE PLASMID RESISTS OTHER PLASMIDS AND PHAGE



# HAFNIA PROPHAGE ENCODING CHEESE FLAVOR GENES?

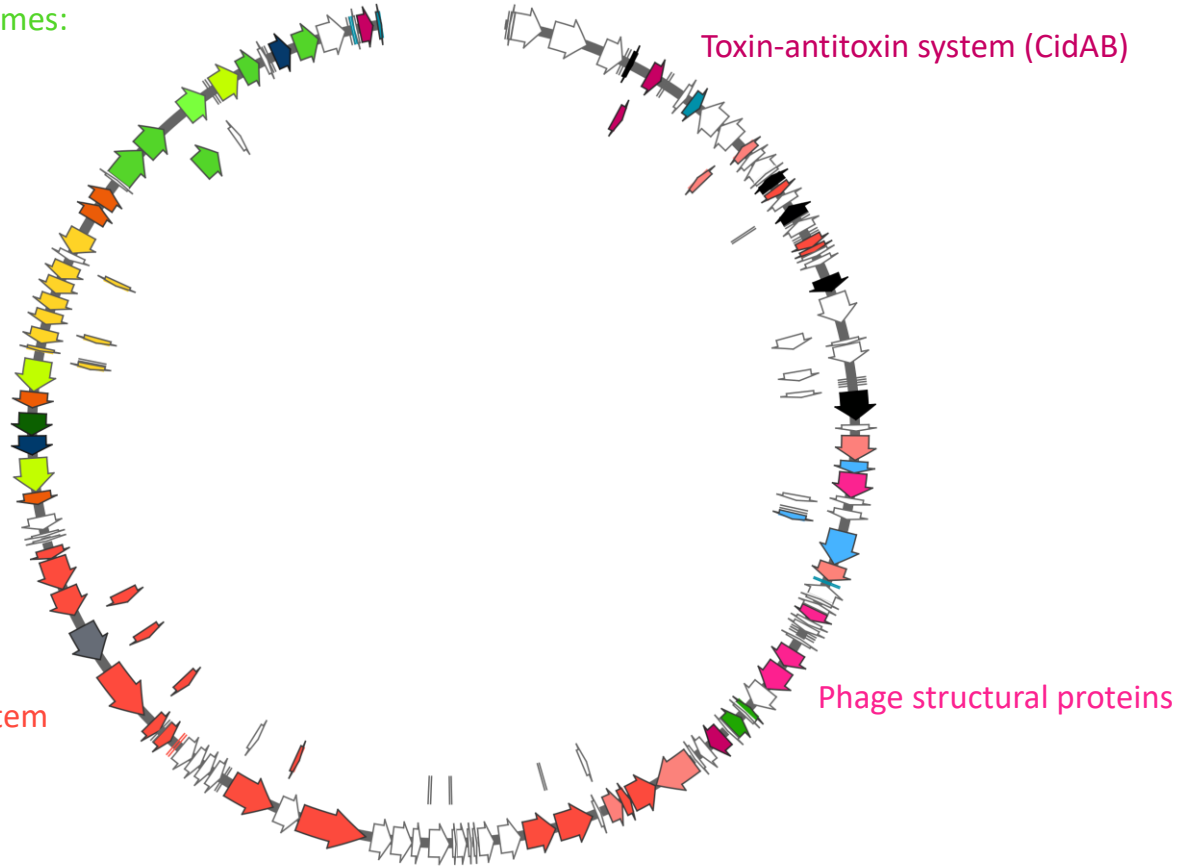
Cheese flavor related enzymes:  
glutaminase and  
methionine gamma-lyase

Cobalt and cobalamin  
transport and  
metabolism

Type VI secretion system

Toxin-antitoxin system (CidAB)

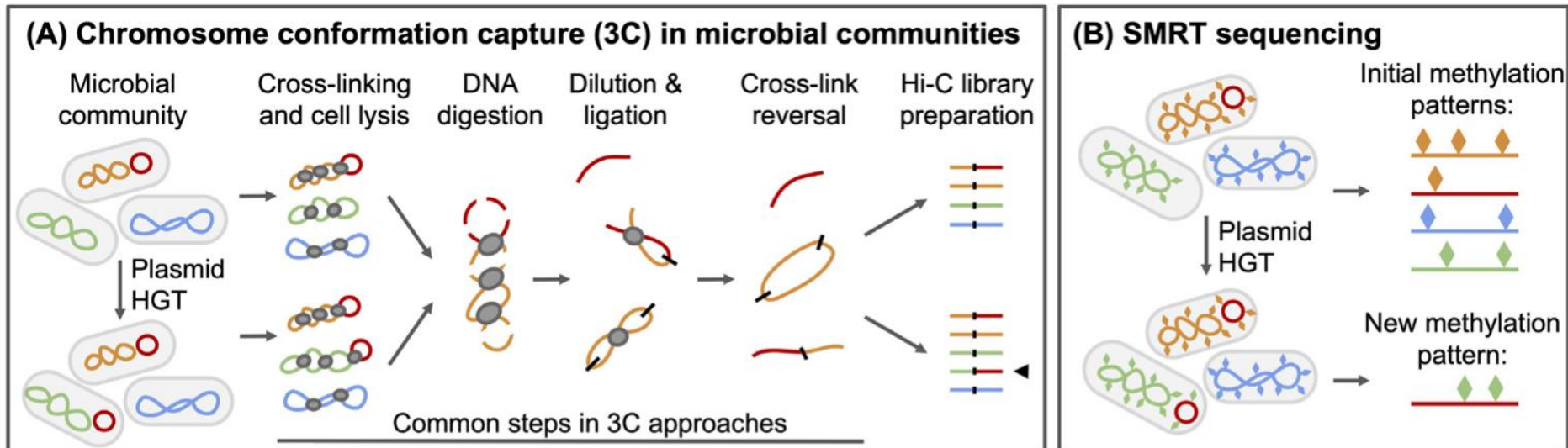
Phage structural proteins



REVIEW ARTICLE

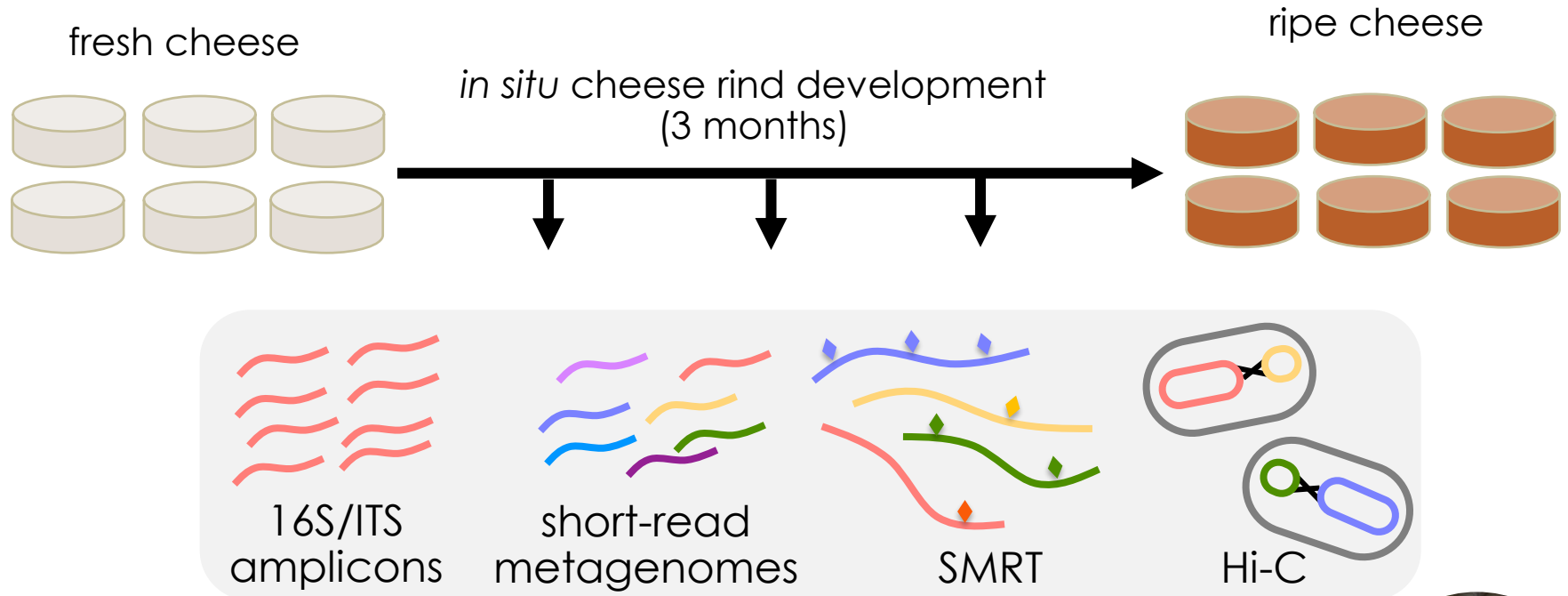
# Experimental approaches to tracking mobile genetic elements in microbial communities

Christina C. Saak, Cong B. Dinh and Rachel J. Dutton\*





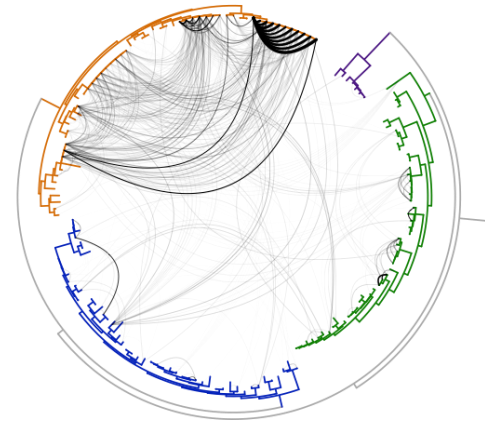
# CAN WE OBSERVE HGT IN CHEESE?



Christina Saak  
Postdoctoral fellow

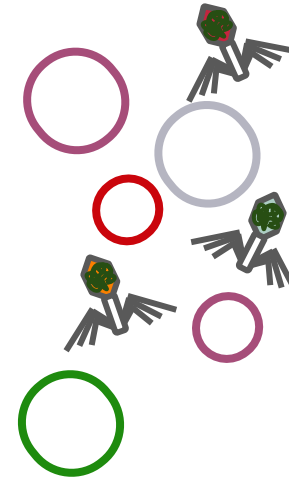
# ABUNDANT EVIDENCE OF HGT IN CHEESE GENOMES

- REPEATED TRANSFER OF IRON UPTAKE PATHWAYS

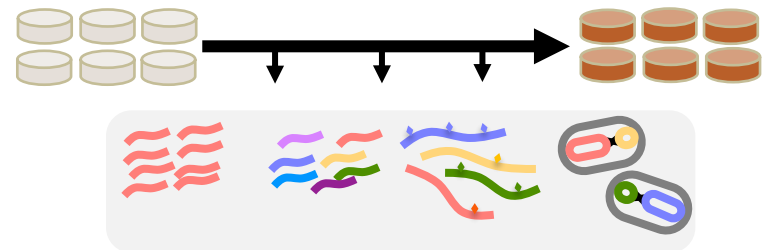


# DIVERSE SET OF PLASMIDS AND PHAGE EVEN IN "SIMPLE" COMMUNITIES

- SO MUCH MORE BIOLOGY TO DISCOVER AND EXPLORE!



# DEVELOPING APPROACHES TO TRACK HGT





# COLLABORATORS

- Laura Sanchez (UIC)
- Benjamin Wolfe (Tufts)
- Nick Loman (U Birmingham)
- Kit Pogliano (UCSD)
- Pieter Dorrestein (UCSD)
- Rob Knight (UCSD)
- Nancy Keller (UW-Madison)
- Sergey Kryazhimskiy (UCSD)
- Michael Harms (Univ of Oregon)

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Graduate students: Brooke Anderson, Cong Dinh, Tara Spencer  
Undergraduates: Brooke Johnson, Angel Sarabia, Martha Herrera  
Lab manager: Steven Villareal

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Undergraduates: Carla Espinoza, Val Jackson-Hundley, Gillian Belk, Antony Yang, Will Bushnell, Tyler Nelson, Kien Malarney, Daniel Rubin, Shanice Webster, Alyson Yee, Rajashree Mishra, Miriam Schiffman, Juan Alvarez, Adriann Negreros

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