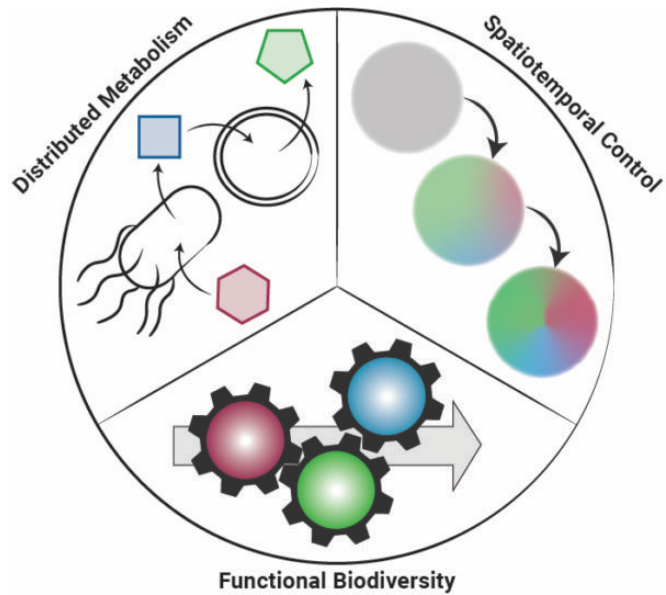


# Environmental Control of Microbiomes

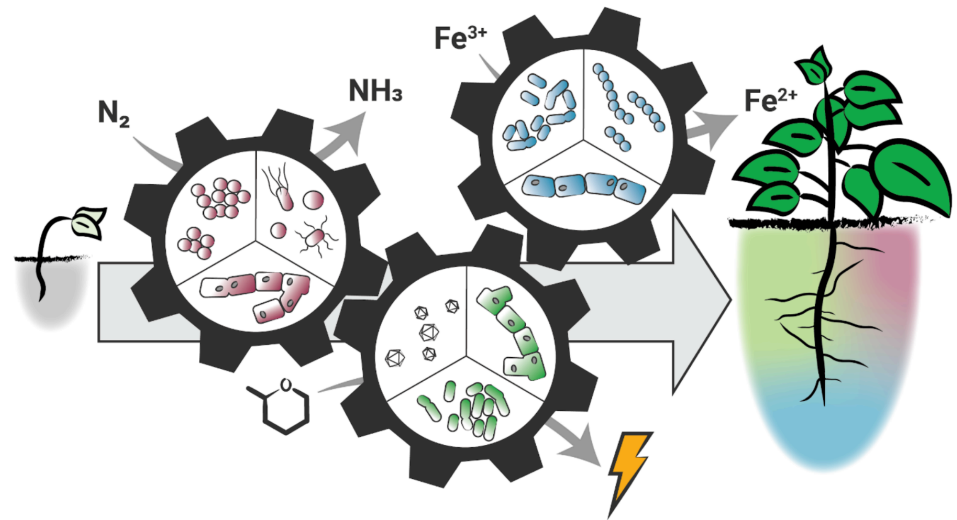
Will Sharpless

Oversight from Drs. Adam Arkin, Kyle Sander, Fangchao Song and Jennifer Kuehl

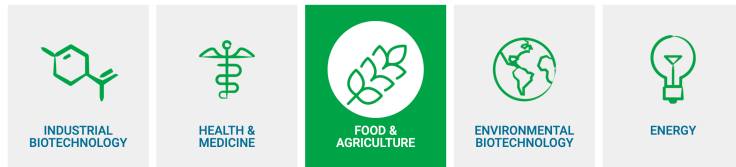
# Overview: Microbiome Engineering



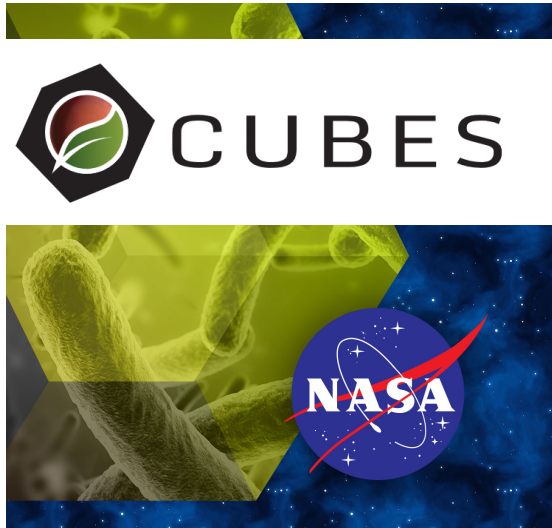
*EBRC's Microbiome Engineering Roadmap*  
(Released October 19<sup>th</sup>, 2020)



Certain organizations of microbes can induce functional phenotypes. Therefore, by controlling diversity, one might control microbiome-caused effects on the host.



## Motivation: NASA CUBES, Enhanced Rice Growth



Center for the Utilization of Biological Engineering in Space, Food & Pharmaceutical Synthesis Division:

*"To utilize plant and microbial engineering to realize food and pharmaceuticals for astronauts along with the recycling of plant wastes"*

With respect to microbiome design, the goal is to find a set of rhizosphere organisms that promote rice growth, form a stable community, inhibit pathogen invasion etc. to support the growth of robust hydroponic rice yields

## **Predicting Microbiomes:** Generalized Lotka-Volterra Model (gLTV)

For an  $n$ -dimensional community, the change in population  $x_i$  can be described,

$$\dot{x}_i = x_i \left( r_i + \sum_{j=1}^n \alpha_{ij} x_j \right) \quad i \in [1, n]$$

Where  $r_i$  is the innate growth rate for species  $i$ , and  $\alpha_{ij}$  is the “interaction” coefficient from species  $j$  to species  $i$ .

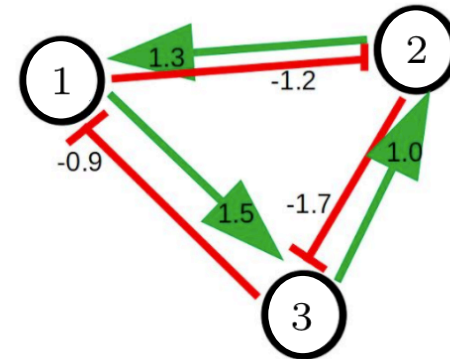


### Eg. 3-member gLV

$$\dot{x}_1 = x_1 r_1 + \alpha_{11} x_1^2 + \alpha_{12} x_1 x_2 + \alpha_{13} x_1 x_3$$

$$\dot{x}_2 = x_2 r_2 + \alpha_{21} x_1 x_2 + \alpha_{22} x_2^2 + \alpha_{23} x_2 x_3$$

$$\dot{x}_3 = x_3 r_3 + \alpha_{31} x_1 x_3 + \alpha_{32} x_2 x_3 + \alpha_{33} x_3^2$$



(Self loops,  $\alpha_{ii}$  omitted)

or in vector form,

$$\begin{pmatrix} \dot{x}_1 \\ \dot{x}_2 \\ \dot{x}_3 \end{pmatrix} = \begin{pmatrix} x_1 & 0 & 0 \\ 0 & x_2 & 0 \\ 0 & 0 & x_3 \end{pmatrix} \left( \begin{pmatrix} r_1 \\ r_2 \\ r_3 \end{pmatrix} + \begin{pmatrix} \alpha_{11} & \alpha_{12} & \alpha_{13} \\ \alpha_{21} & \alpha_{22} & \alpha_{23} \\ \alpha_{31} & \alpha_{32} & \alpha_{33} \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \\ x_3 \end{pmatrix} \right)$$

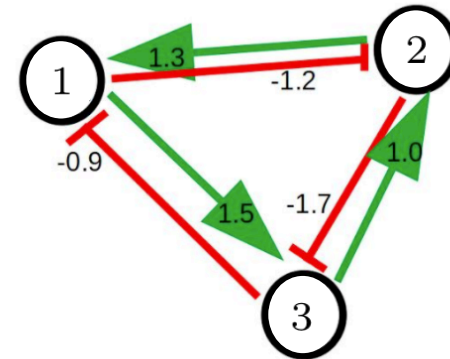
$$\dot{x} = \text{Diag}(x)(r + Ax)$$

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$$\dot{x} = \text{Diag}(x)(r + Ax)$$

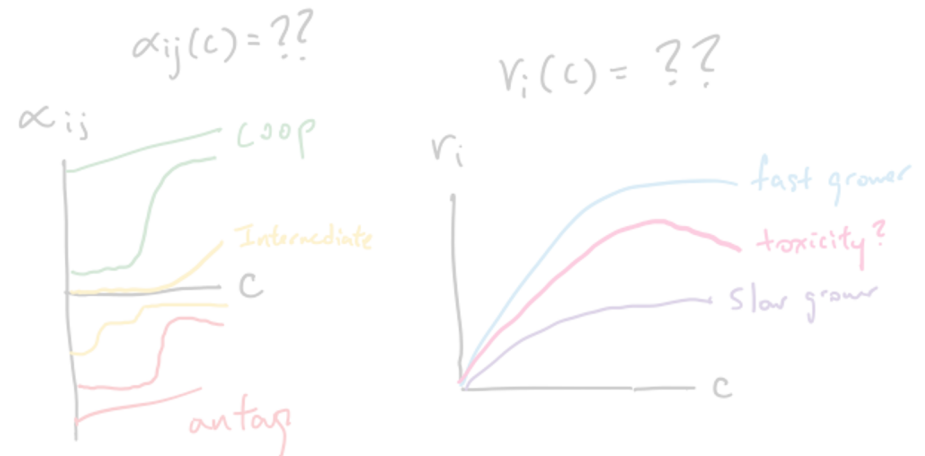
**Hypothesis:** Growth Rates and Interactions depend on the Environment, and thus, the dynamics of the community do as well

We might consider a concentration and gLV model,

$$\dot{x} = \text{Diag}(x)(r + Ax)$$

$$\dot{c} = -\Gamma x - \text{Diag}(\gamma_c)c + u$$

$$x, r \in \mathbb{R}^n \quad A \in \mathbb{R}^{n \times n} \quad c, u, \gamma_c \in \mathbb{R}^s \quad \Gamma \in \mathbb{R}^{s \times n}$$



Explicit understanding of these functions,  $A(c)$  &  $r(c)$  would describe how to modulate the environment for the most desirable community topology.

Additionally, it describes how the dynamics and stability of the community vary with environment.

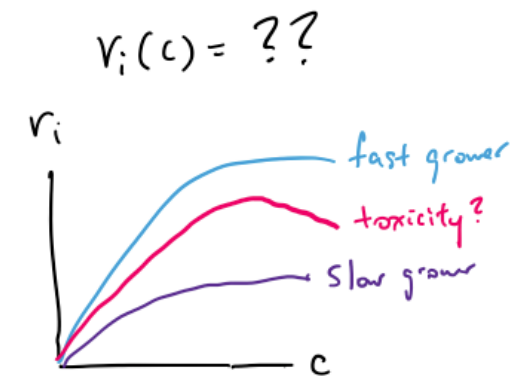
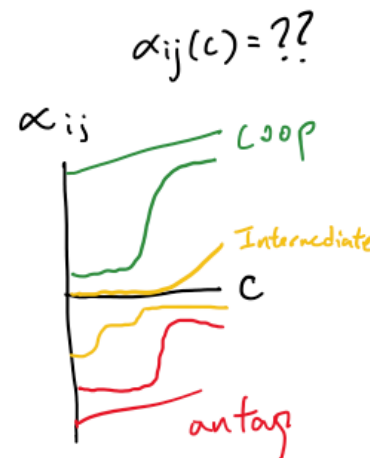
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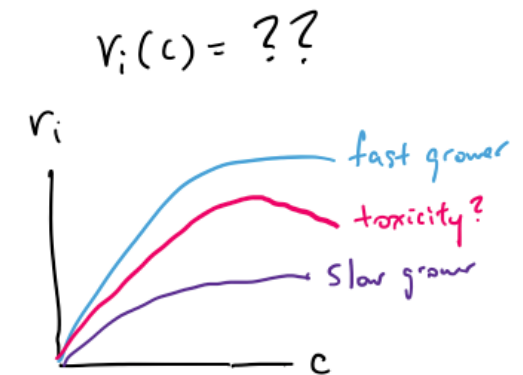
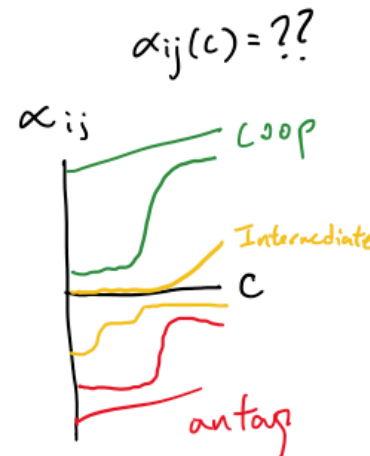
**Hypothesis:** Growth Rates and Interactions depend on the Environment, and thus, the dynamics of the community do as well

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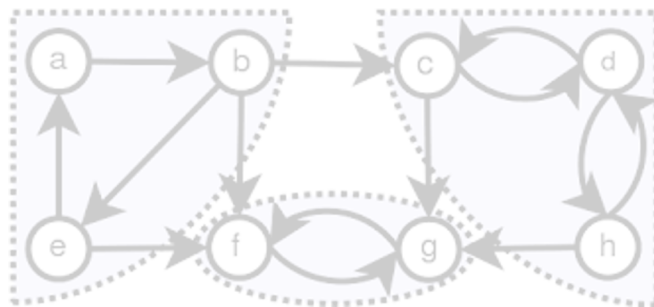
## Purpose: Community Design wrt the Environment

Note, if  $A$  and  $r$  are functions of the environment ( $c$ ) then any nontrivial community equilibrium  $x^*$  and its stability (not shown) is a function of environment ( $c$ ),

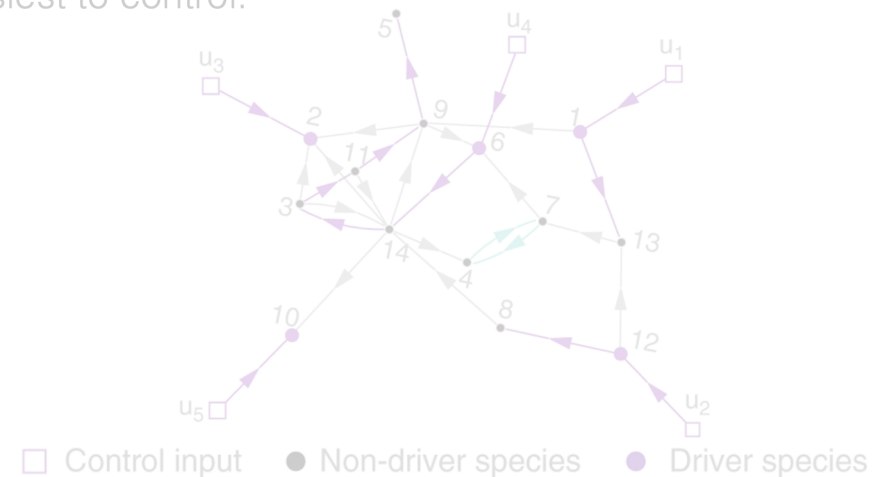
$$\text{Nontrivial equilibrium } x^* \iff Ax^* = -r$$

Hence, by controlling  $c$  we might choose an equilibrium and its stability best suited for the community purpose.

Furthermore, the minimum number of species needed to drive the community state depends on the edges of the directed graph, the  $A$  [Liu et al. 2019]. Thus, we might use  $c$  to pick the network which is spanned by the fewest SCC's, to make the community easiest to control.



*Strongly connected components (SCC).*



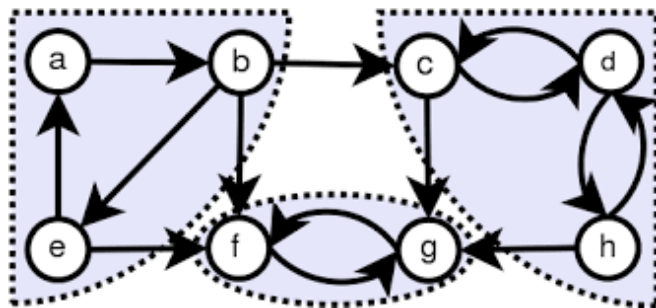
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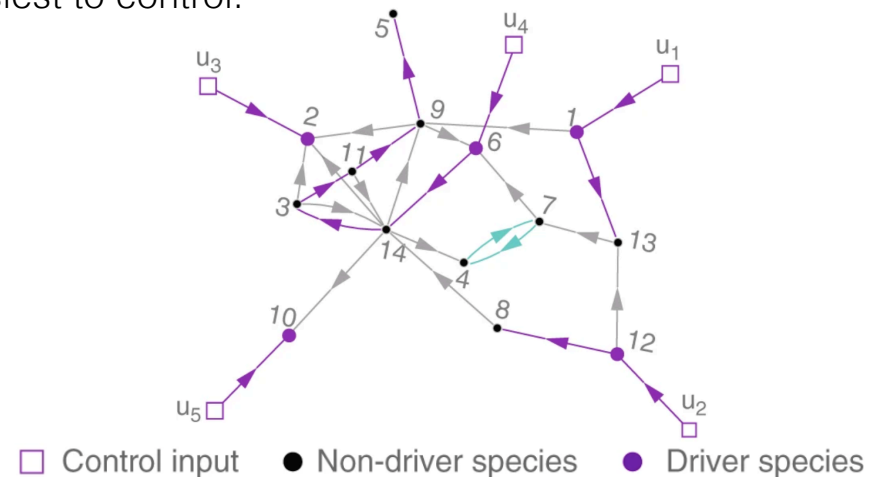
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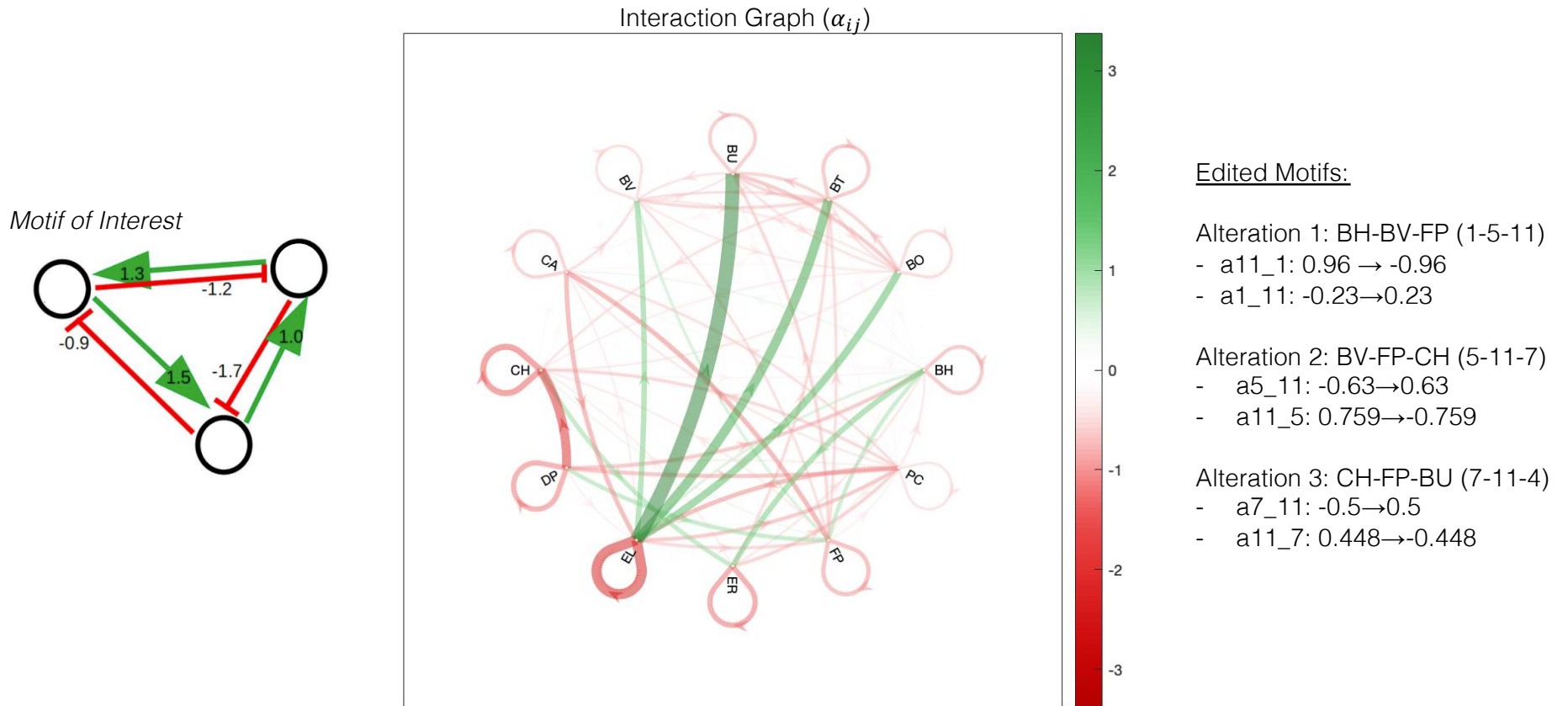
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*Strongly connected components (SCC).*



## Demonstration: Variations in two interactions



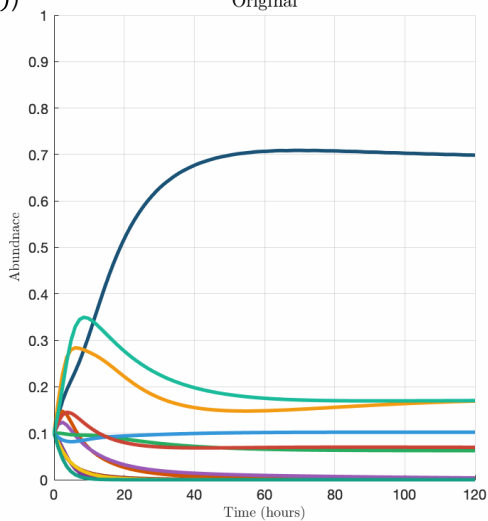
Parameterized mouse-gut community by former lab member Dr. Ophelia Venturelli  
(Venturelli et al. 2019)



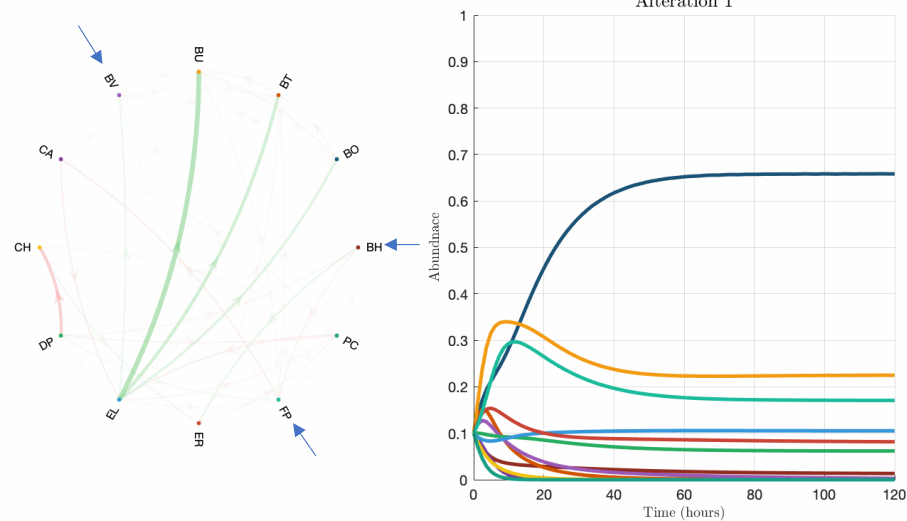
“Active” Intrxn-Pop Graph ( $\alpha_{ij}x_i(t)x_j(t)$ )

### Community Graphs and Trajectories at Time 1

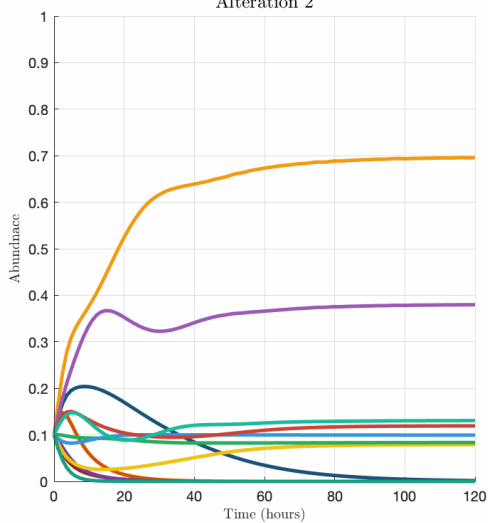
Original



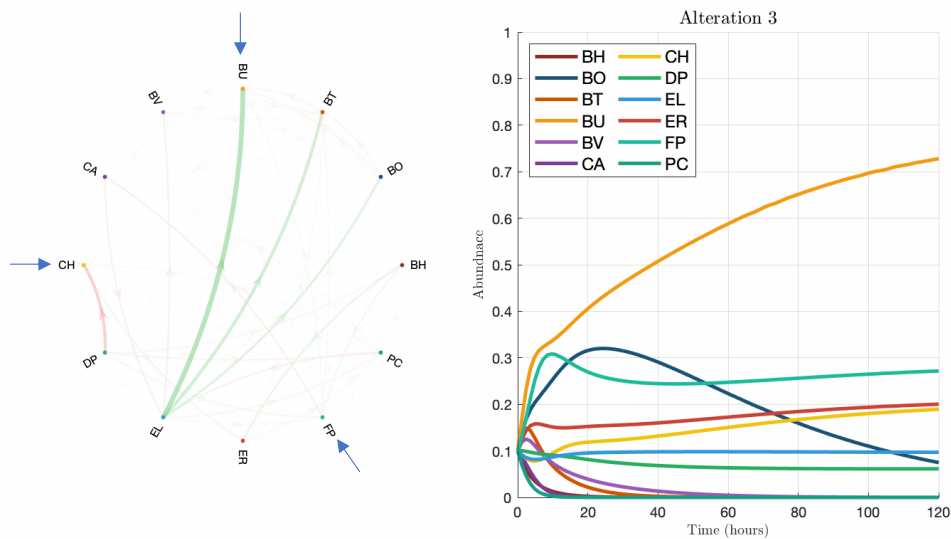
Alteration 1



Alteration 2

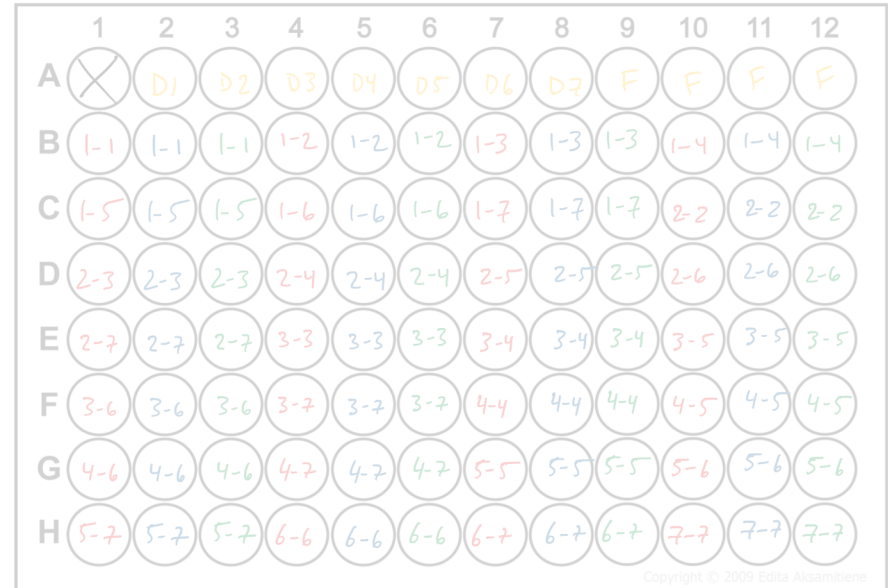


Alteration 3

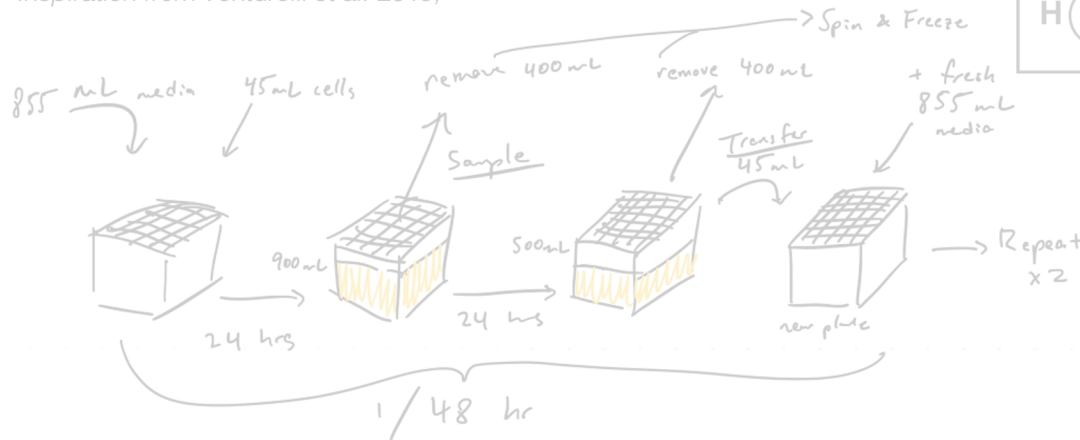


# Method: How to ascertain gLV parameters; pairwise growth culturing

Closest Related to Isolate	PW Id	Soil-Plant Source	Inspiration for Inclusion
<i>Agrobacterium salinitolerans</i>	99.0%	Mojave Roots	<a href="https://link.springer.com/article/10.1007%2Fs12275-010-0082-1">https://link.springer.com/article/10.1007%2Fs12275-010-0082-1</a>
<i>Shinella kummerowiae</i>	97.0%	Mojave Roots	<a href="https://www.ncbi.nlm.nih.gov/pubmed/18523187">https://www.ncbi.nlm.nih.gov/pubmed/18523187</a>
<i>Microbacterium phyllosphaerae</i>	98.6%	Roots and Root-Associated calcine clay soil - Uninoculated	<a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6210106/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6210106/</a>
<i>Pseudomonas koreensis</i>	98.1%	Regolith Grown Plant Roots	<a href="https://www.sciencedirect.com/science/article/abs/pii/S1049964412000771">https://www.sciencedirect.com/science/article/abs/pii/S1049964412000771</a>
<i>Bacillus megaterium</i>	99.5%	Mojave Roots	<a href="https://www.annualreviews.org.libproxy.berkeley.edu/doi/full/10.1146/annurev.micro.62.081307.162918">https://www.annualreviews.org.libproxy.berkeley.edu/doi/full/10.1146/annurev.micro.62.081307.162918</a>
<i>Panotea agglomerans</i>	98.6%	Res Bulk Soil	<a href="https://www.ncbi.nlm.nih.gov/pubmed/30743637">https://www.ncbi.nlm.nih.gov/pubmed/30743637</a>
<i>Flavobacterium ginsengiterrae</i>	83.5%	Roots and Root-Associated calcine clay soil - Uninoculated	<a href="https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-017-1117-0">https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-017-1117-0</a>



Inspiration from Venturelli et al. 2019,



Replication along environmental gradient (in TSB)

Glucose concentration gradient (6 plates)

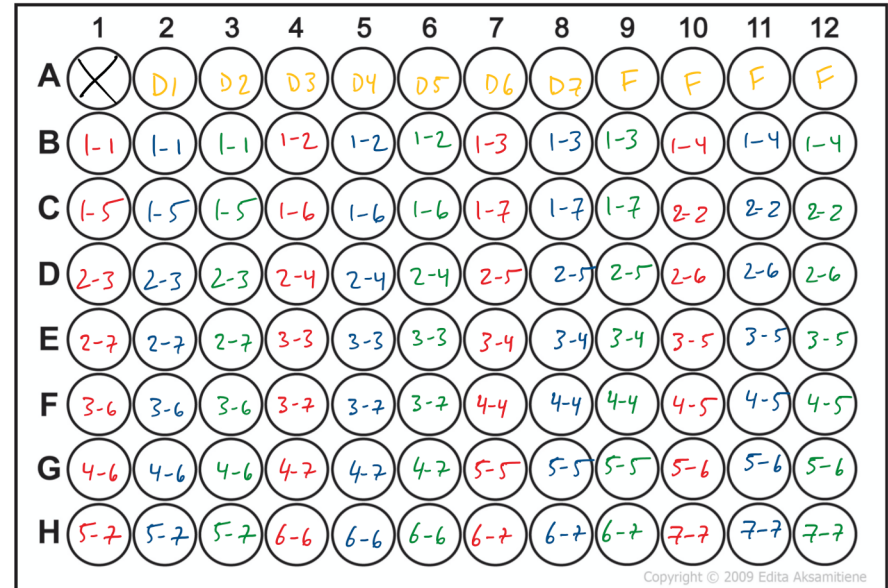
- 0, 0.31, 1, 3.1, 10, 31 mM
- Assume that microbial strategy doesn't vary in 2 days

Temperature gradient (5 plates)

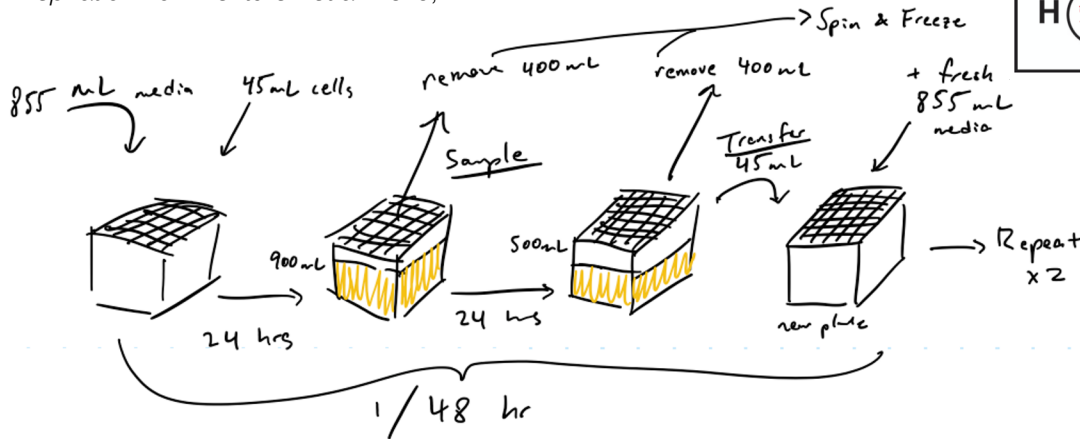
- 22.5, 25, 27.5, 30, 32.5 degrees C

# Method: How to ascertain gLV parameters; pairwise growth culturing

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Glucose concentration gradient (6 plates)

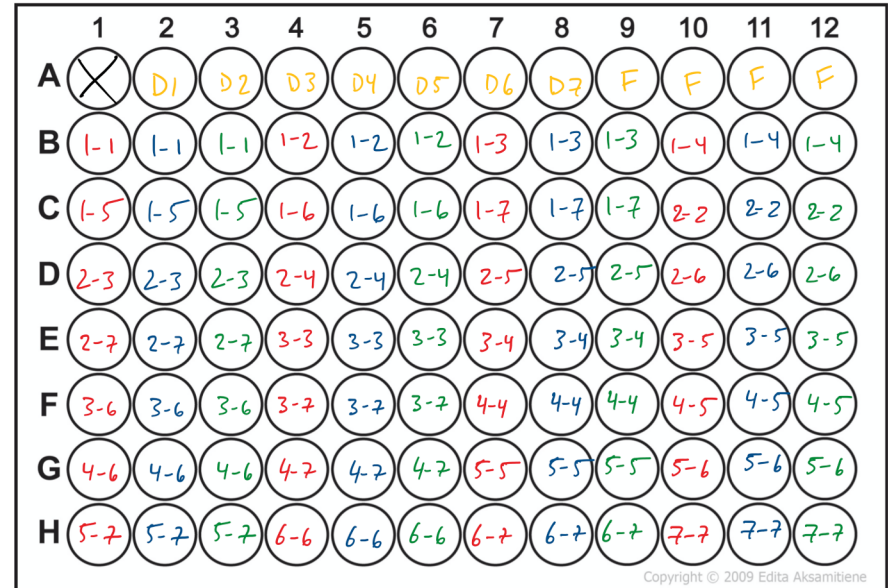
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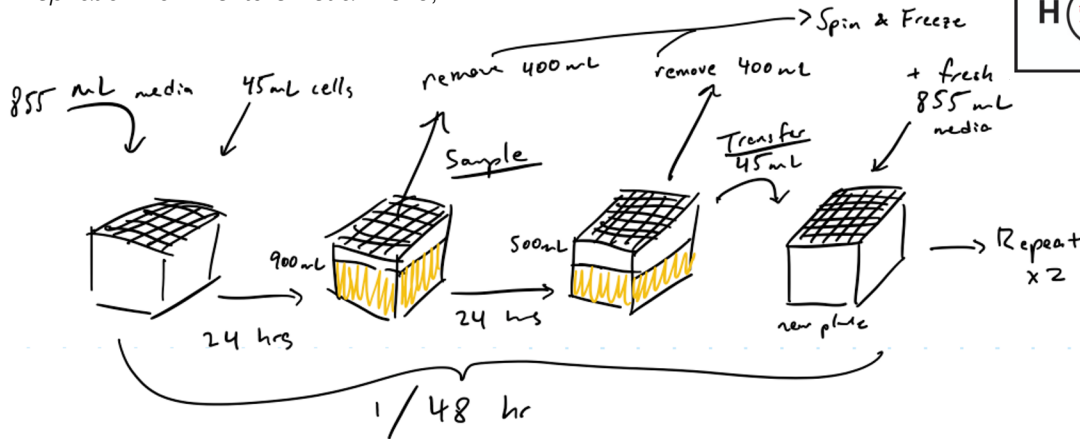
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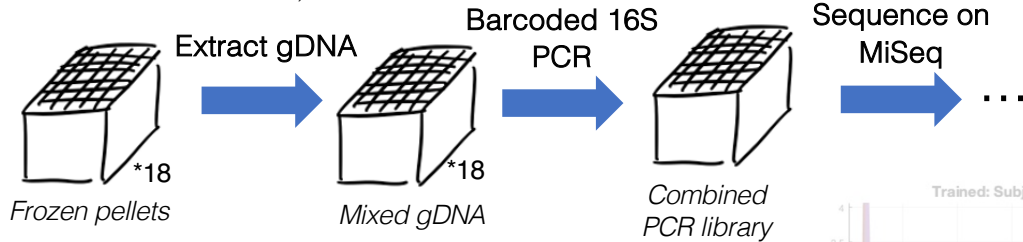
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Temperature gradient (5 plates)

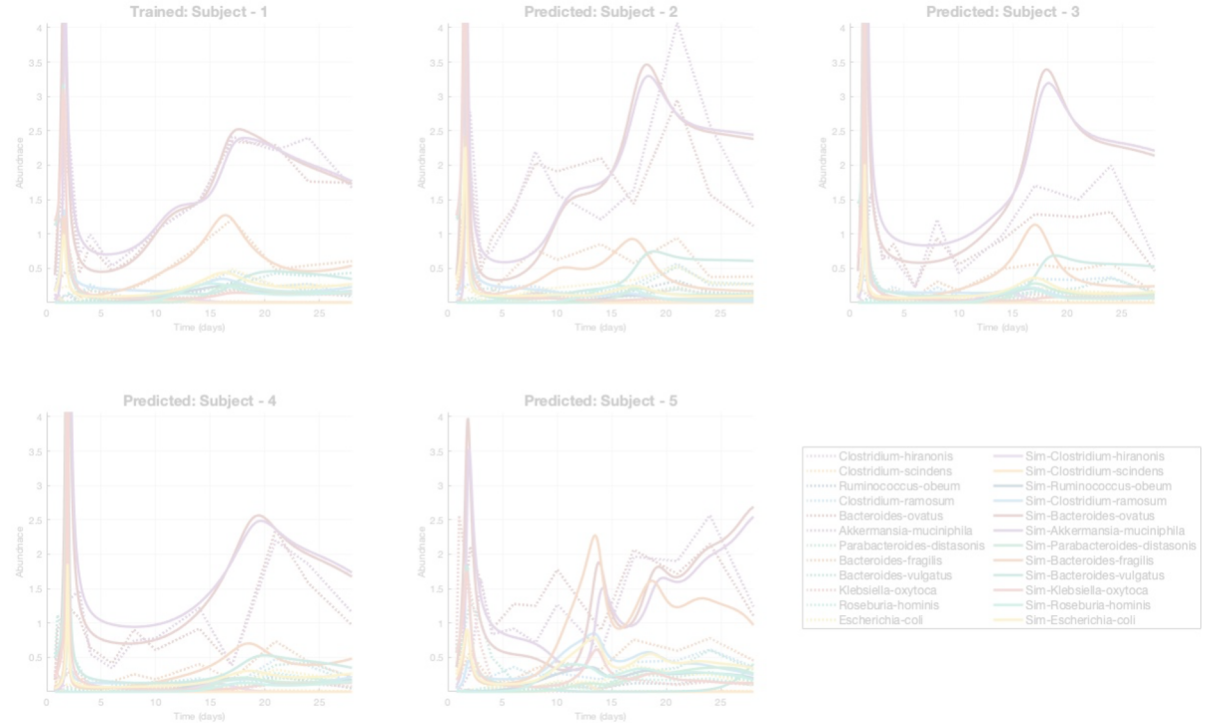
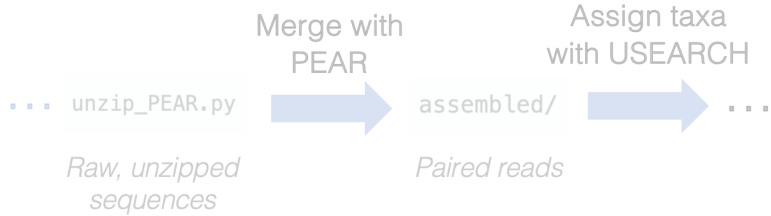
- 22.5, 25, 27.5, 30, 32.5 degrees C

# Method: How to ascertain gLV parameters; back end

From Venturelli et al. 2019,



Parameterization with MATLAB: minimize sum squared norm of predicted and actual trajectories

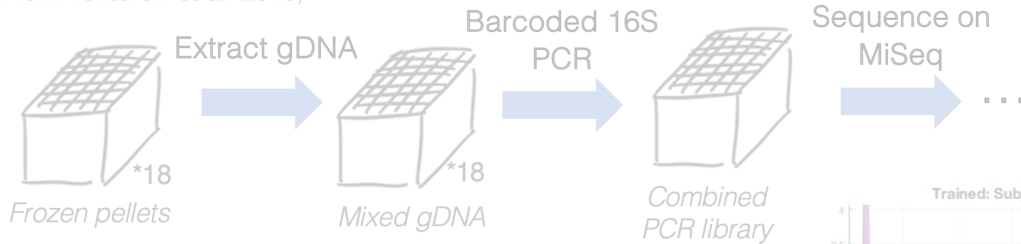


Utilizing mouse gut data from MDSINE study (Bucci et al. 2016)

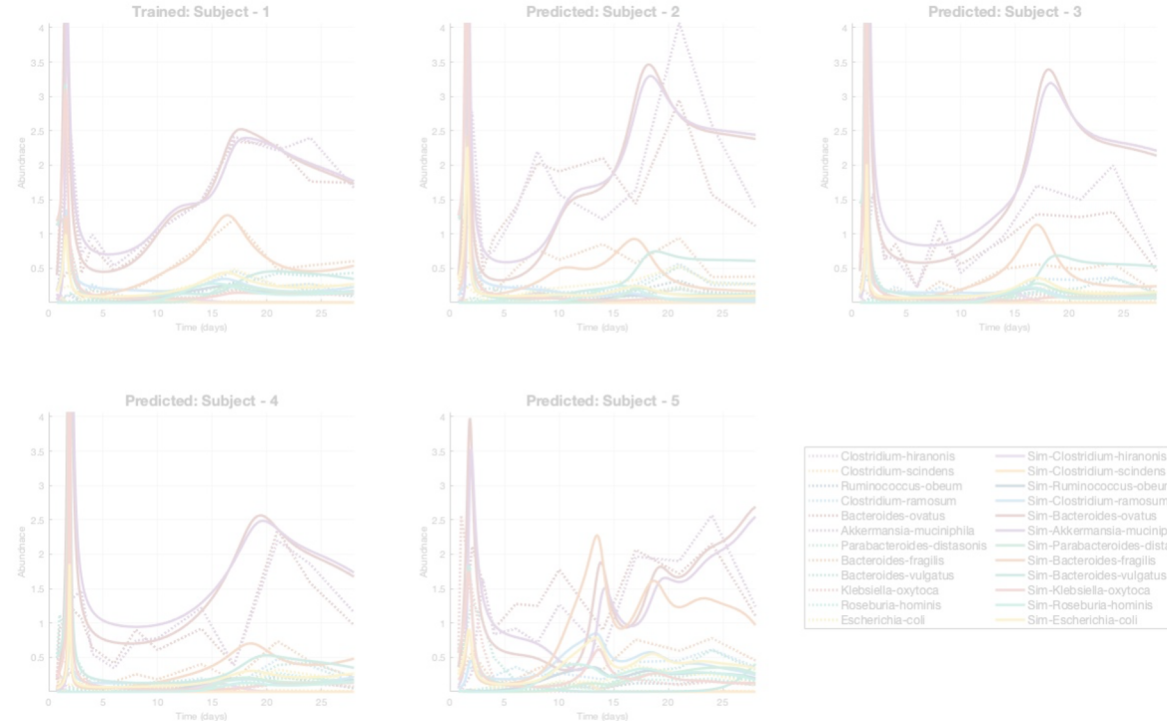
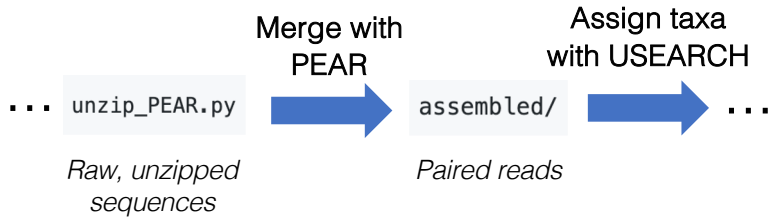


# Method: How to ascertain gLV parameters; back end

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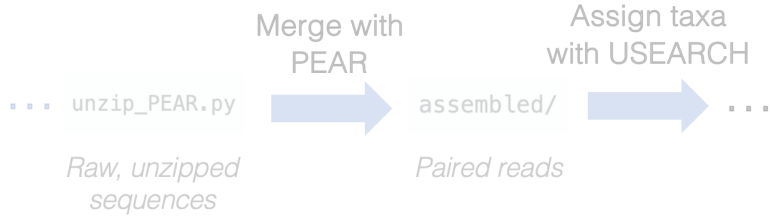
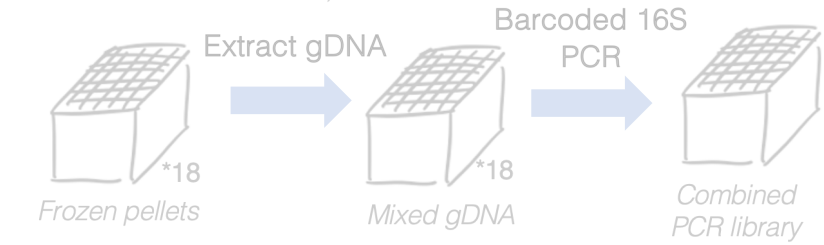
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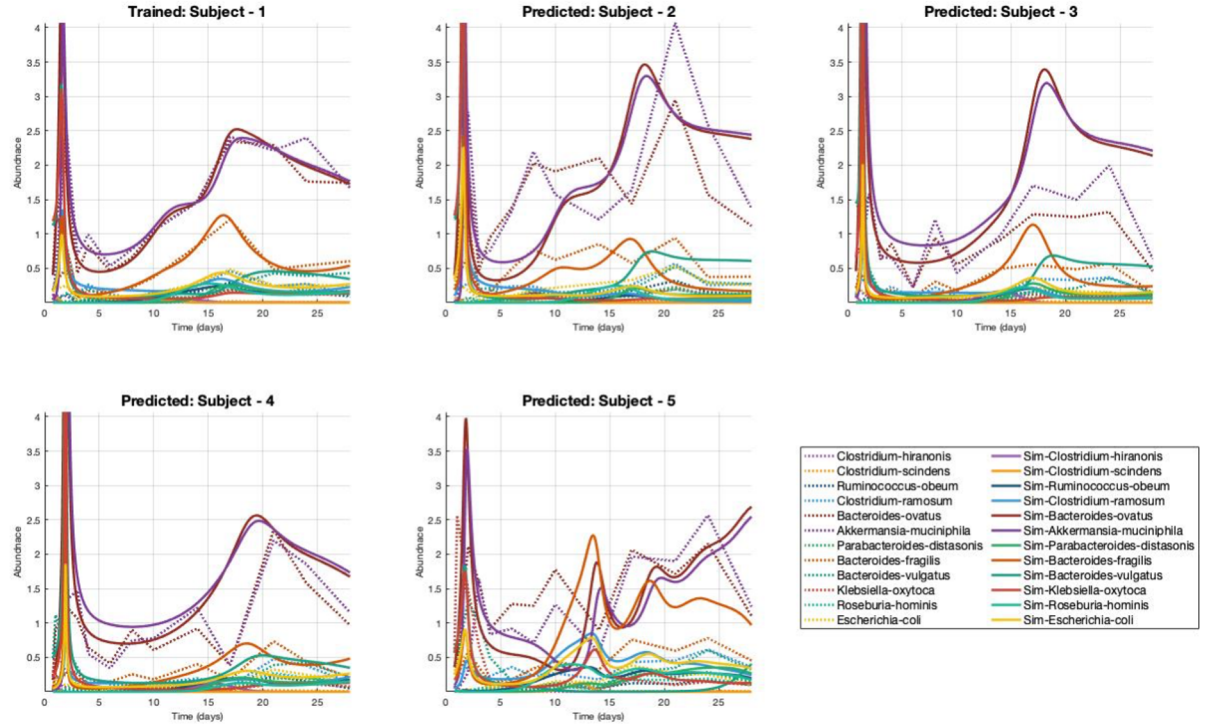
From Venturelli et al. 2019,



counts.txt + OD Data taken throughout the experiment  
Sorted species counts



Parameterization with MATLAB:  
minimize sum squared norm of predicted and actual trajectories



Utilizing mouse gut data from MDSINE study (Bucci et al. 2016)

# Progress

## Done

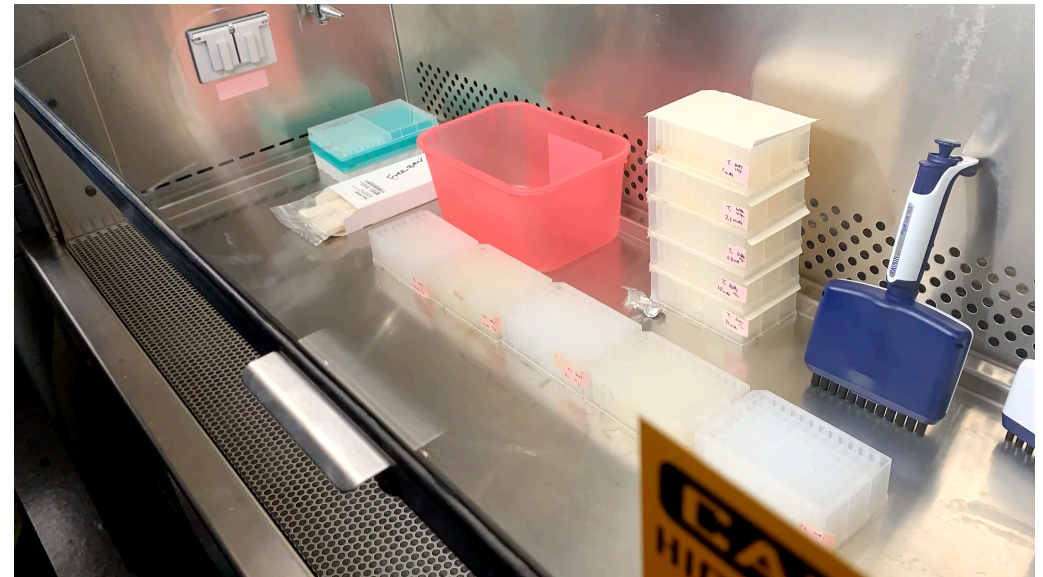
- Growth rate sampling to tune concentration range to realistic growth for hydroponic system.
- Full growth experiment with 7 member community
- Adapted and validated oldsequencing pipeline
- Biomek protocol outlined for gDNA extractions
- Wrote and validated parameterization code

## Still Need to

- gDNA extraction, and standardize
- Parameterize and elucidate environmental dependence
- Summarize work for peer-reviewed publication
- Library PCR, cleanup and sequence
- Check parameters predict community cultures

## Future Work

- Design and test a Model Predictive Controller to suggest which concentrations and temperatures a given community should be passaged into to track a desired state trajectory





# **Thank you!**

Thank you Adam, Kyle, Fangchao, Gwyneth, Patricia and others for directly mentoring my research, and CNR for providing me this awesome opportunity.