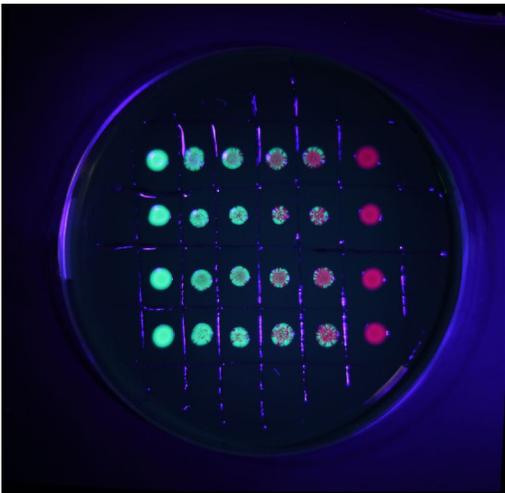
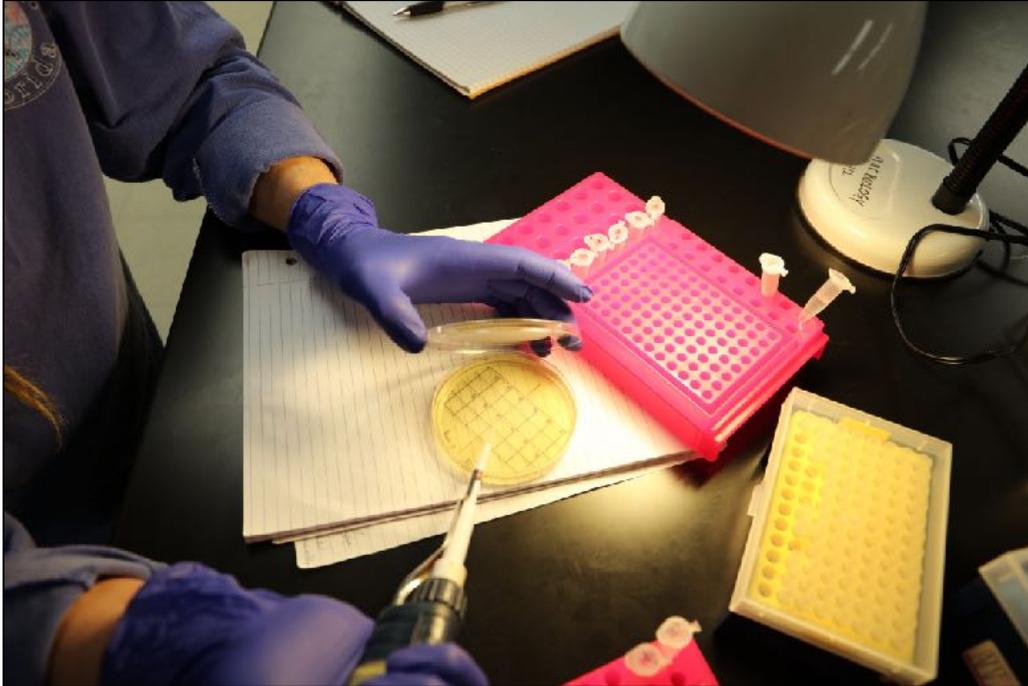
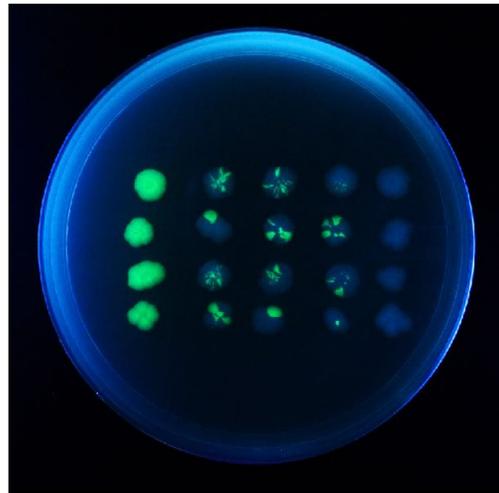


Composing Through Sectoring: A Lab Protocol, Art/Research Assay Project

ARC/BAC collaborators Dr. Ken Kubo and Carolyn Angleton developed a series of microbial experiments to facilitate sectorized colony growth. These protocols seek to control pattern development in bacterium by selectively varying: Species combinations, cell dilutions, distributive ratios, aliquot size, culture media, supplemental growth factors, and incubation temperature and time.



CRISPR red/CRISPR green



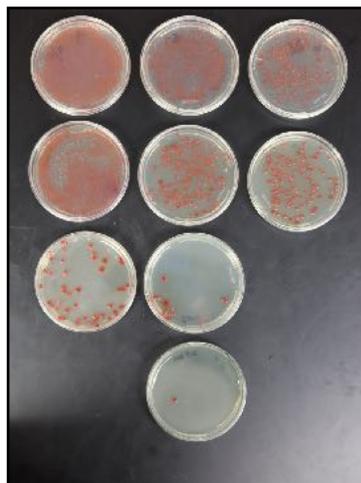
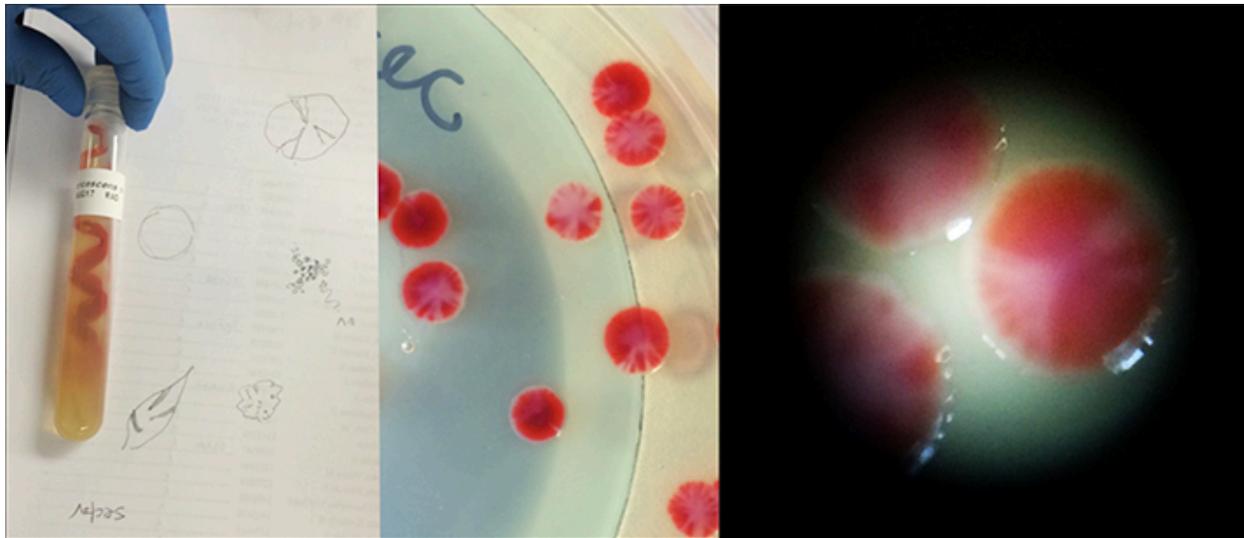
Gfp/Bfp

Sectoring Assay: *Serratia marcescens*

Experimental Design: By exposing *S. marcescens* to UV light, attempt to mutate the bacteria and create differential cell lines in order to affect sectoring patterns.

Protocol:

1. Inoculate tube containing LB media with *S. marcescens* from freezer stock. Grow overnight.
2. Do a serial dilution and plate to look for density of growth, size of colonies and to obtain single colonies.
3. Select a single colony, make liquid culture and grow overnight.
4. Using a micropipette, place 100uL of liquid culture into center of plate. Using a flame jar, metal spreader and turntable, spread bacteria and grow upside-down in 38 degree incubator overnight.
5. Set up a system with a UV light source and timer that will allow you to expose a plate to short durations of UV light. (0-5 sec)
6. While maintaining sterile conditions, take lid off and expose plate with *S. marcescens* to UV light.
7. Assess sectoring patterns by eye and with microscope. (You get different information about cell lineage and colony morphology at the different visual levels)



Red, White, and Sectored Colonies

Sectoring Assay:

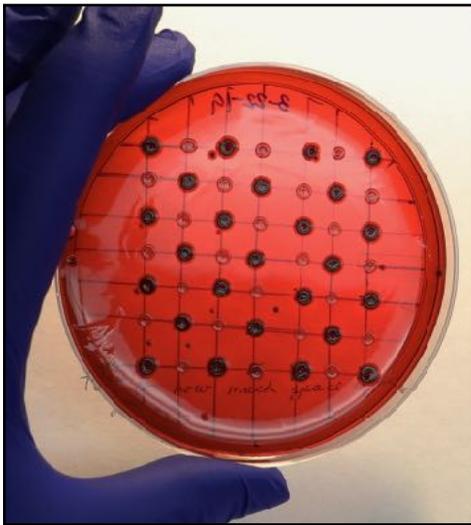
Lac Z Operon

Lac + /Lac - bacteria

Experimental Design:

Protocol:

1. Plate and determine size of inoculation that will work. Evaluate growth parameters
2. Plate on EMB and MacConkey agar (MacConkey agar is a indicator, a selective and differential culture media designed to isolate Gram-negative bacilli and differentiate them based on lactose fermentation. EMB (Eosin methylene blue) agar is a selective and differential medium that inhibits Gram positive organisms)
3. Contemplate and appreciate bacterial beauty-> Lac + color on EMB media is metallic green
4. Create dilution mixes and perform a sectoring assay on LB media: Lac + on left, Lac - on right. Note color changes and whether both species are present. Evaluate experimental variables in terms of future protocol development



Aliquot Size Test



EMB and McConkey Agar
Selective and Differential Media



Lac + on EMB Media

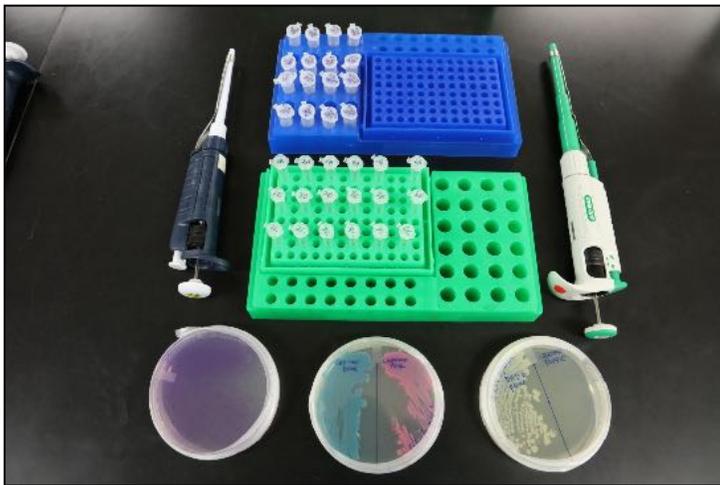


Sectoring Assay:

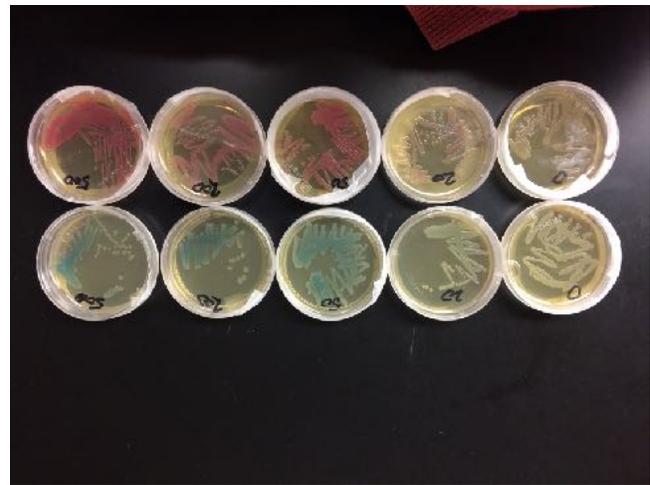
Chromobacterium

Protocol

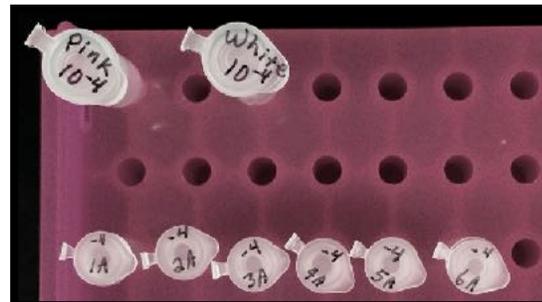
1. Make broth culture from freezer stock of bacteria samples
2. Streak pink, turquoise, purple chromobacteria and use *S. aureus* for white
3. Test for color production w various levels of IPTG
3. Do a serial dilution of each color
4. Combine cells in a proportional assay
5. Inoculate agar plates in a grid structure
6. Grow for two days in 38 degree incubator
6. Assess cell density, looking for contiguous cells (cells touching)
-> If cells are not contiguous, there will be no sectoring



Chromobacteria samples, cell dilution tubes, pipettes

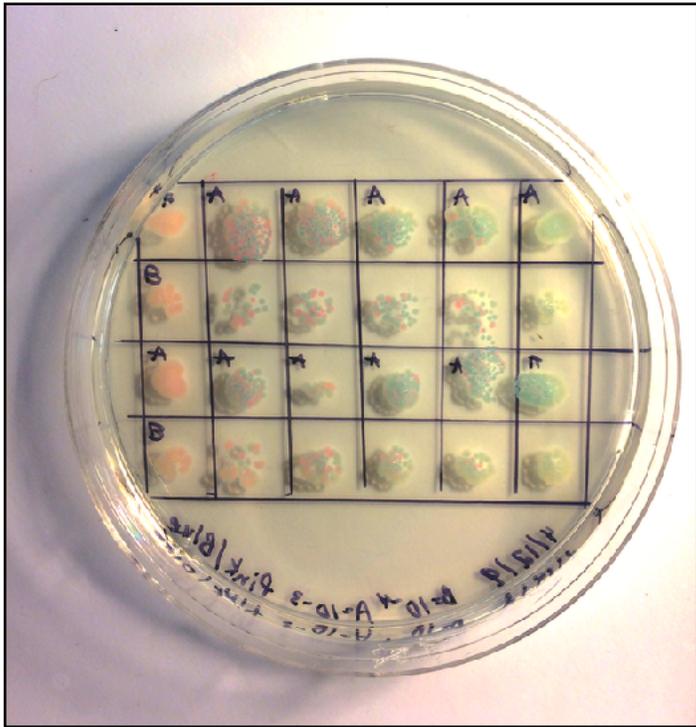


IPTG tests for color strength

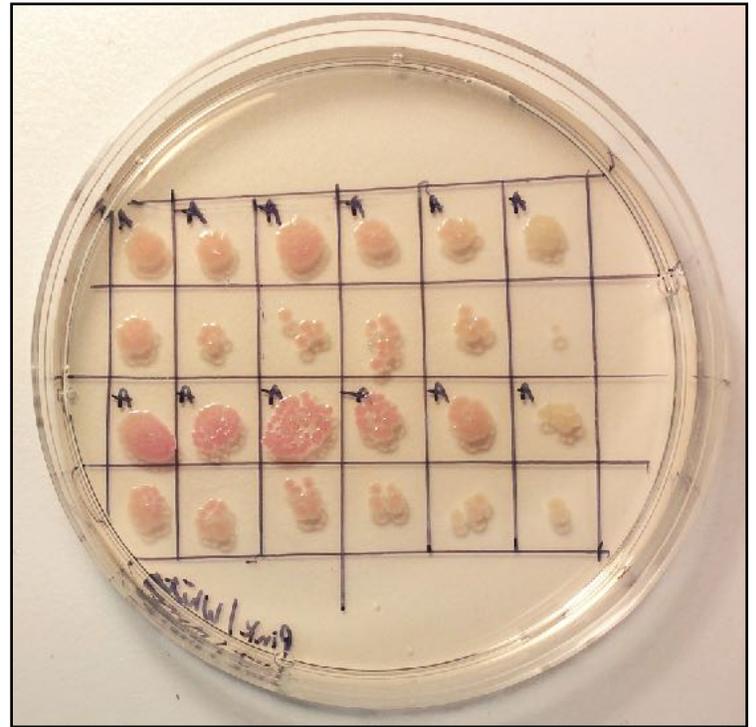


10 (-4) dilutions of pink and white

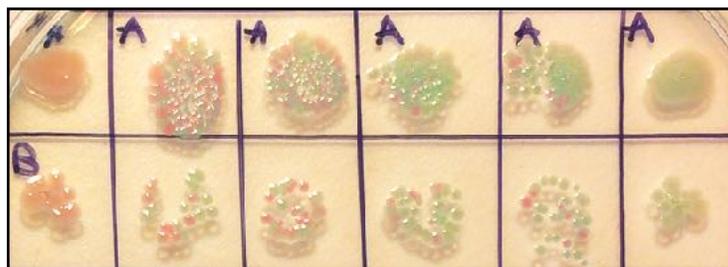
Chromobacterium Results:



Blue/Pink Chromobacteria

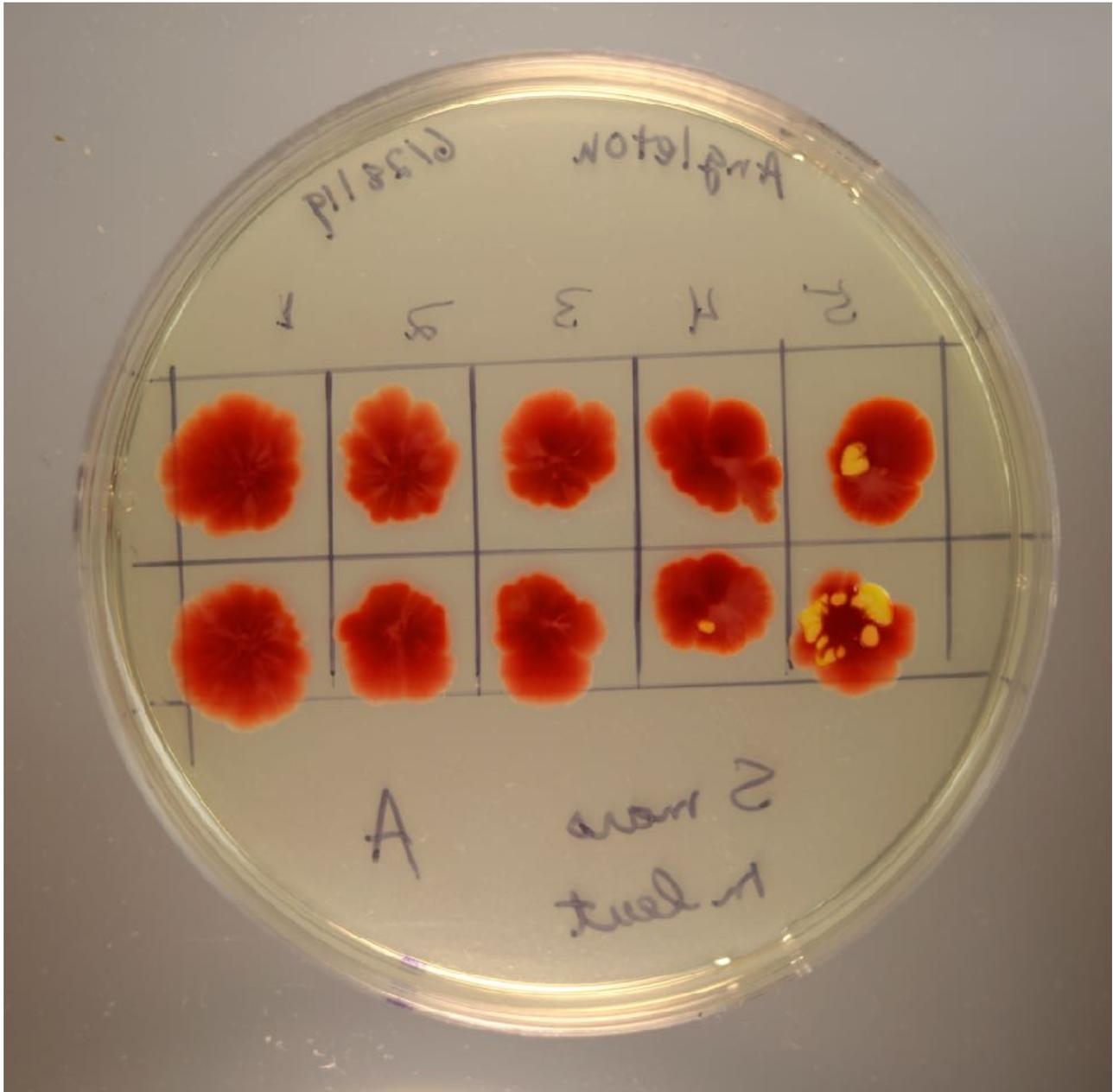


Pink Chromo/ White

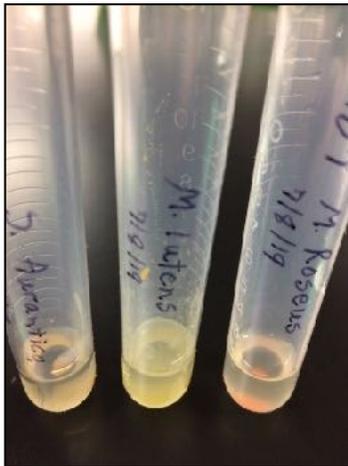


Noncontiguous cells: cell density too low to create sectoring

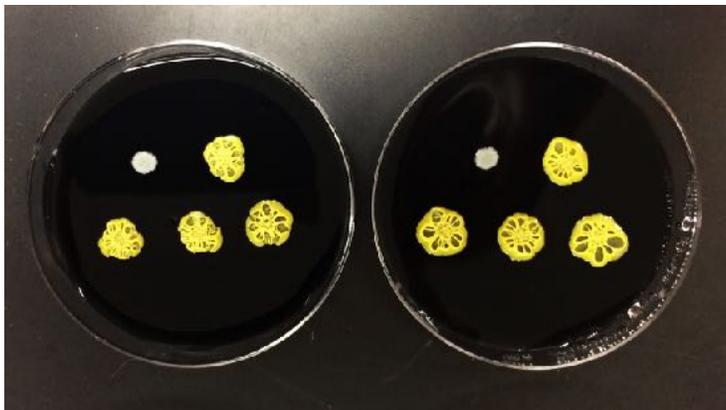
Sectoring Assay:
Gram (+) vs. Gram (-) Bacteria
S. marcescens* / *M. luteus



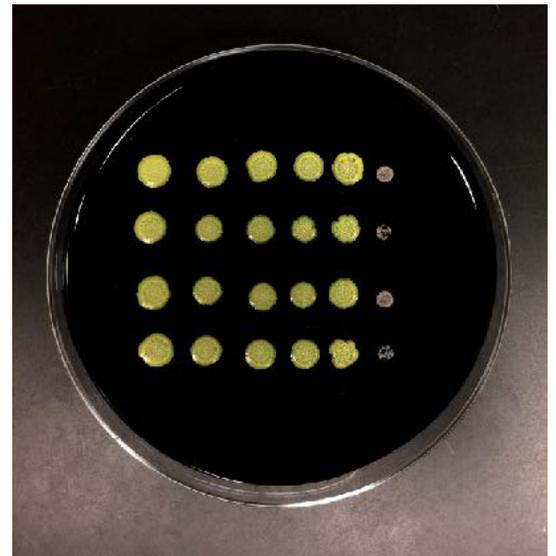
**Sectoring Assay:
Gram Positive Pigmented bacteria**



S. aurantiaca, *M. luteus*, *M. roseus* in tube cultures and on streak plates



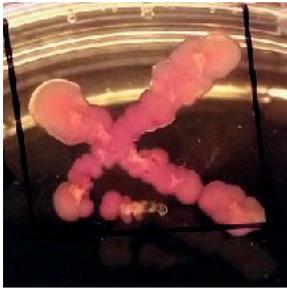
Sectoring Assay: *S. aurantiaca*/ *M. luteus*



Sectoring Assay: *M. luteus*/ *M. roseus*

Sectoring Assay:

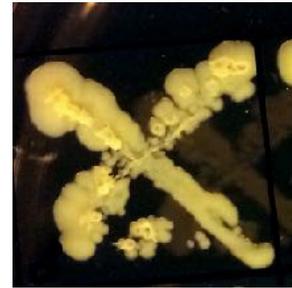
CRISPR *E. coli* Endogenous and Edited transformants:



E. coli w Rfp in endogenous genome



CRISPR *E. coli* in a partially edited state

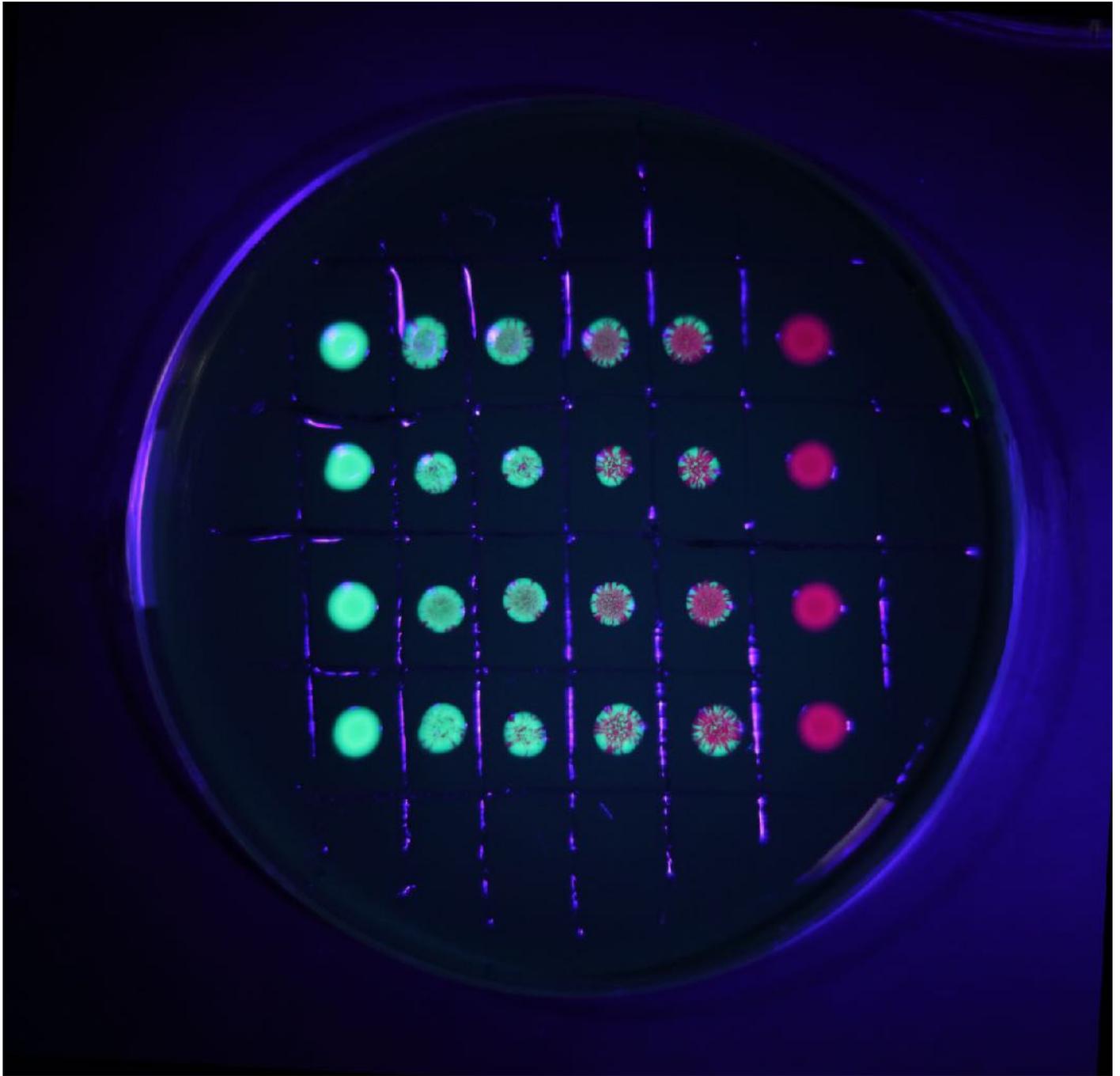


E. coli w CRISPR inserted Gfp gene synthesizing new protein

CRISPR Red, CRISPR Green



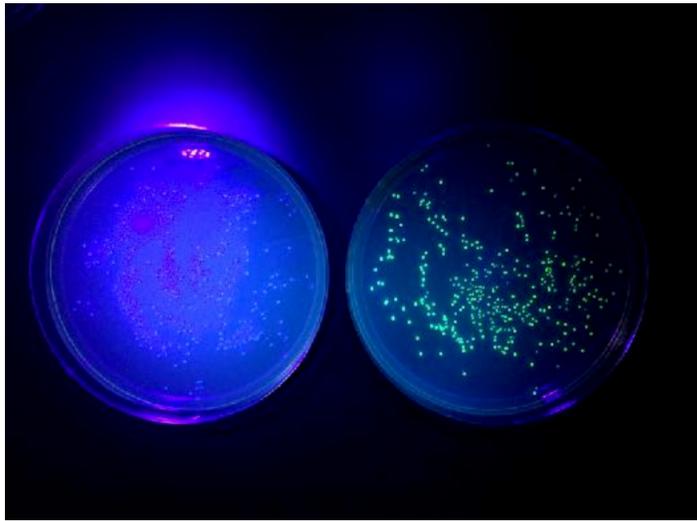
Streak plate w red Rfp control



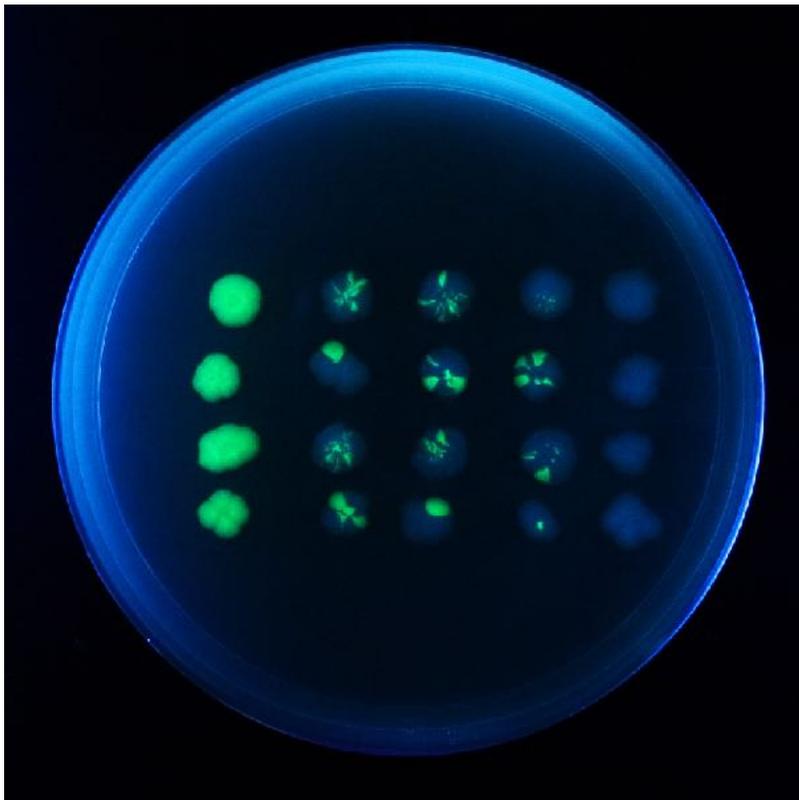
CRISPR Red, CRISPR Green Sector Plate on Black Agar

Sectoring Assay:

E. coli mutated w Green Fluorescent Protein and Blue Fluorescent Protein



Gfp, Bfp *E. coli* plated individual colonies



GFP/BFP Mutagenesis Sector Plate (Mutagenesis series designed by ARC/BAC collaborator Dr. Adam Telleen)