# Metagenomic screening of environmental samples for novel biosensors

**Arhaan Gupta-Rastogi[1,2], Scott Gaines[2], James Galagan[2]**

Sacred Heart Preparatory, 150 Valparaiso Avenue, Atherton, CA 94027, Galagan Lab, Department of Biomedical Engineering, Boston University College of Engineering, MA 02215

## Introduction

**Background**:

- Metagenomics, the comprehensive study of genetic material recovered directly from environmental samples, has revolutionized our understanding of microbial communities.
- Highlights that a vast majority of microbes are unculturable with untapped genomes.
- Building upon a study that demonstrated the capability of specific bacteria to degrade the steroid hormones, we introduced a cocktail of hormones—progesterone, testosterone, and estrogen— expecting to see higher change in GFP

**Goal**:

- Investigate the potential of metagenomics to uncover proteins responsive to steroids, which could be pivotal in biosensing applications and environmental management.
- The presence of environmental steroids suggests that certain bacteria might possess proteins capable of degrading compounds like testosterone, thereby influencing microbial community dynamics and biochemical processes.
- Create a DNA library using the environmental sample
- Contribute to the Galagan Lab by creating a DNA library and screening it so that it can be used to discover novel enzymes or pathways for steroid production

## Methods

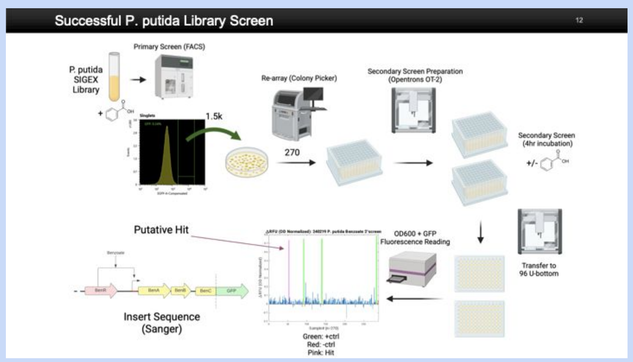


Figure 1: Image of the process

Environmental Sample prep:
- Add LB and carbenicillin
- Carbenicillin is used as an antibiotic because cells without the it will not survive the rest of the test/experiment

Primary Screening:
- Selecting cells with high fluorescence in the condition with a certain steroid cocktail
- Use a FACS machine to measure the fluorescence of the cells
- Sorting samples made from day 0
- Downside is you can only test one condition at a time

Secondary Screening:
- Compare what the cell line will do with and without the steroids to make sure we are collecting specific steroids that aren't always "on"
- Follow up to see if there is differential expression

**Day 1: Primary Screen**

Start ~9am

1) Prepare LB w/ Carbenicillin (LB+ABX)
2) Record the OD600 of the overnight growths
   a. In a cuvette add 400ul fresh LB and 200uL overnight. Real OD = 3x reading
3) Prepare **two** 1mL samples of OD600 = 0.4, diluting overnights w/ fresh LB+ABX into 14mL Nunc tubes
4) Add 10uL DMSO to one of the tubes for each cell line
5) Add 10uL substrate to the other tube for each cell line
6) Incubate tubes at 250rpm at 30C for 4 hrs
7) Wash cells for a total of 2 times
   a. Centrifuge cells at 4000g for 3 min
   b. Decant supernatant
   c. Resuspend in 1mL PBS
   d. Repeat
8) Transfer 200uL of PBS+cells into 1mL of PBS in microcentrifuge tube
9) Record/sort on FACS
   a. Use SIGEX template or previous experiment
   b. For sorting, use Single Cell or Ultra Purity and >1.5k events
   c. If plating, sort into PBS (1.5kb in 500uL PBS), spread 100uL/petri dish. Grow overnight at 30C
   d. If doing a serial screen, sort into 5mL LB+ABX and grow overnight at 250rpm at 37C. Repeat Day 1 Primary Screen procedure

Figure 2: Image of the primary screening protocol

## Results

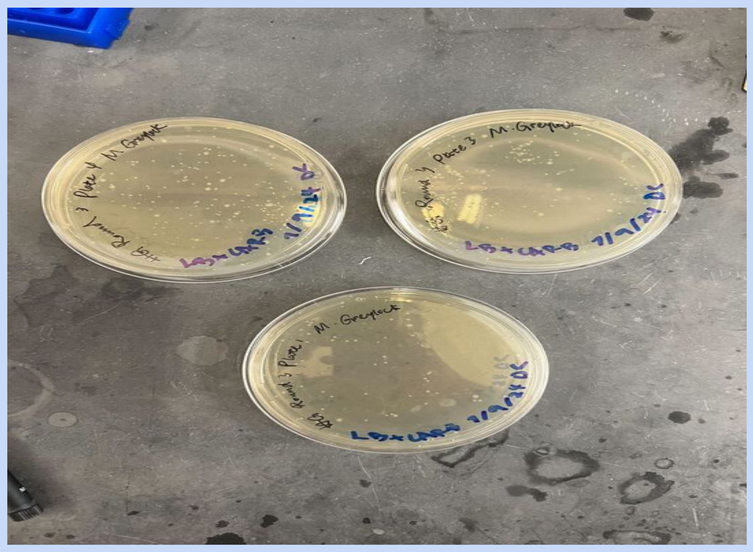

Figure 2. Amount of cells on agar plates increased

- The amount *E. coli* on the plate increased when put in 37 degrees Celsius overnight
- Each cell on the plate is *E. coli* mixed with different inserts from the library, each is now considered a colony
- Selected for difference fluorescence based on what we sorted for
- Plated colonies have plasmids containing carbenicillin resistance
- Isogenic colonies: The colonies all encode different things
- Plasmids contain inserts from the gDNA library and those inserts should encode for potential successful proteins

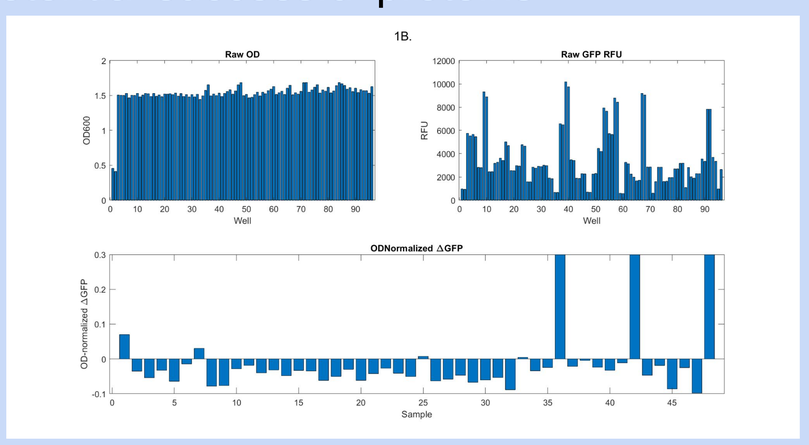


Figure 3. Secondary Screening results

- In the figure, the raw OD is around 1.5
- In the figure, the raw raw GFP fluctuates between 2000 and 12000 RFU
- The spikes in the change in GFP correspond to the positive controls, all other bars are showing that the GFP in the cells did not change with steroids treatment
- This means that the no successful protein was found in the steroid condition
- The tall bar graphs in the bottom right show positive control tests

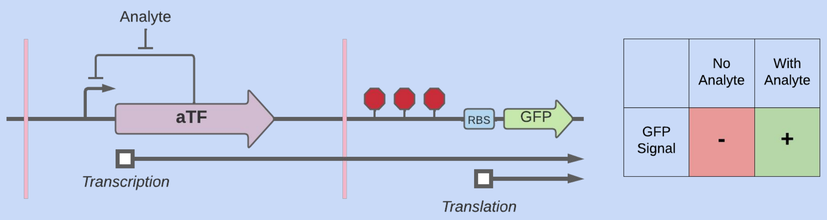


Figure 4. Operon Trap diagram

## Discussion/ Conclusions

Screening Description:
- FACS is a powerful tool to screen many candidate genes at once to filter libraries
- Secondary Screening is used to validate hits

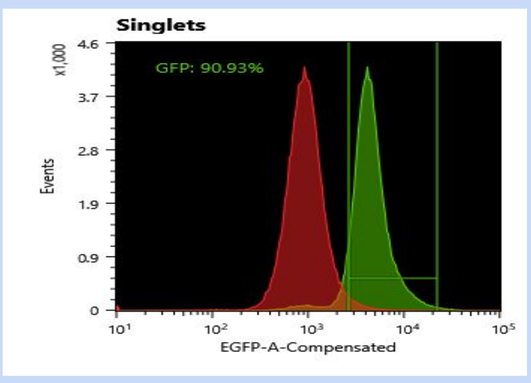


Figure 5. EdcR with cocktail ( green) and no cocktail ( red)

Details:
- TF's are auto inhibitory which bind upstream onto their own promoter which inhibits transcription
- Results in less GFP created
- The protein binds to DNA and steroid binds to protein at allosteric site
- When the steroid binds at this site it makes protein change shape which increases transcription
- This makes GFP a good indicator as it is directly related to if the steroid is working on an aTF

## References

- Luis Angel Ortiz: High-throughput functional screening of oxidase enzymes
- Taku Uchiyama: Substrate-induced gene expression (SIGEX) screening of metagenome libraries

## Acknowledgements

Thank you to Dr. Galagan and Scott, Dr. Tierrafría, Kewei Ye, and all the other staff at Galagan Lab. I appreciate you explaining everything in the lab and answering all of my questions.