



CELLFIEFUGE

ABSTRACT

The biotechnology industry has boomed over the past decade, spearheaded by innovation driven start ups. However, infrastructure constraints have plagued the industry in terms of financial feasibility. Our project aims to cut infrastructure requirements for biomanufacturing initiatives by engineering bacteria that can autoinduce protein overexpression and replace centrifugation.

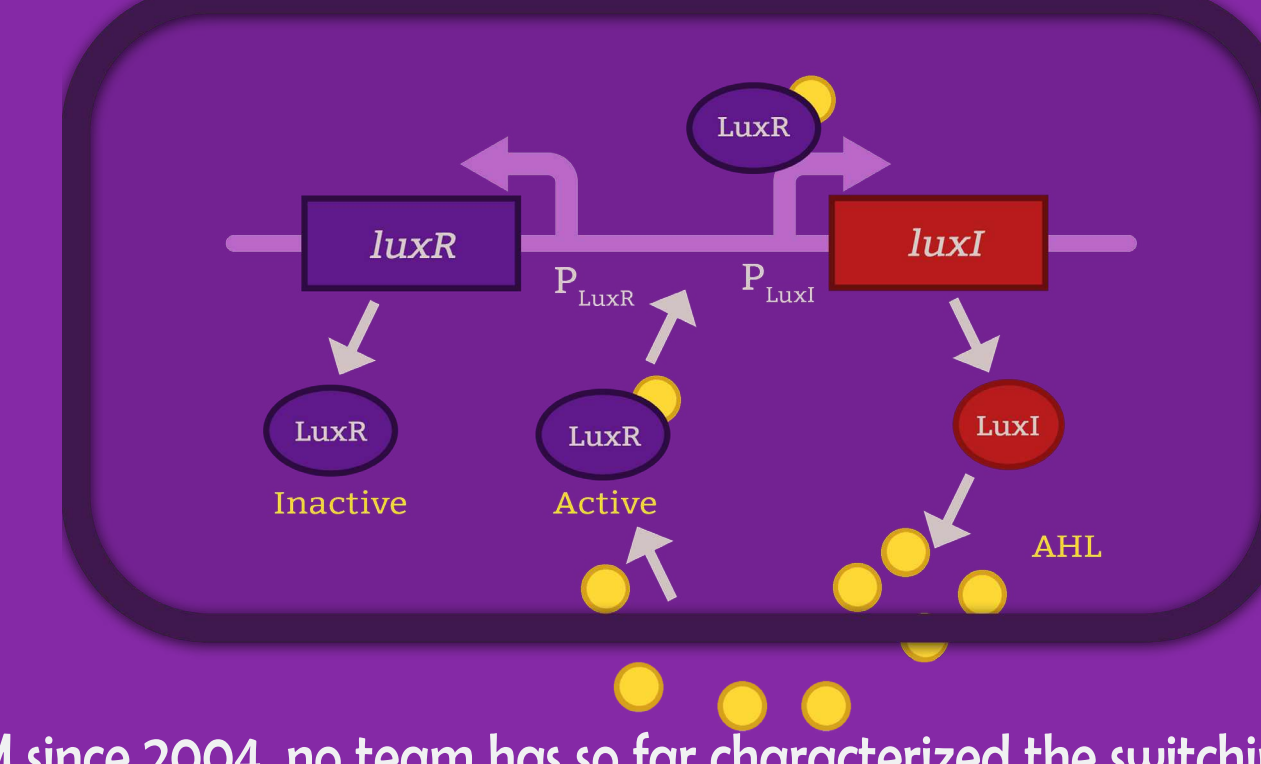
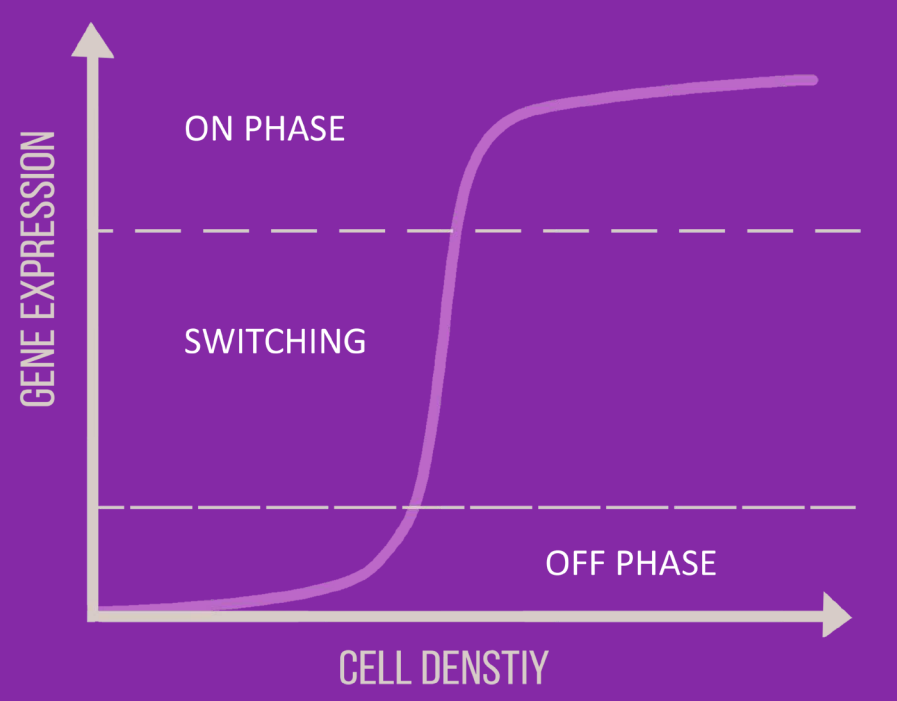
MOTIVATION

Recombinant Protein Synthesis

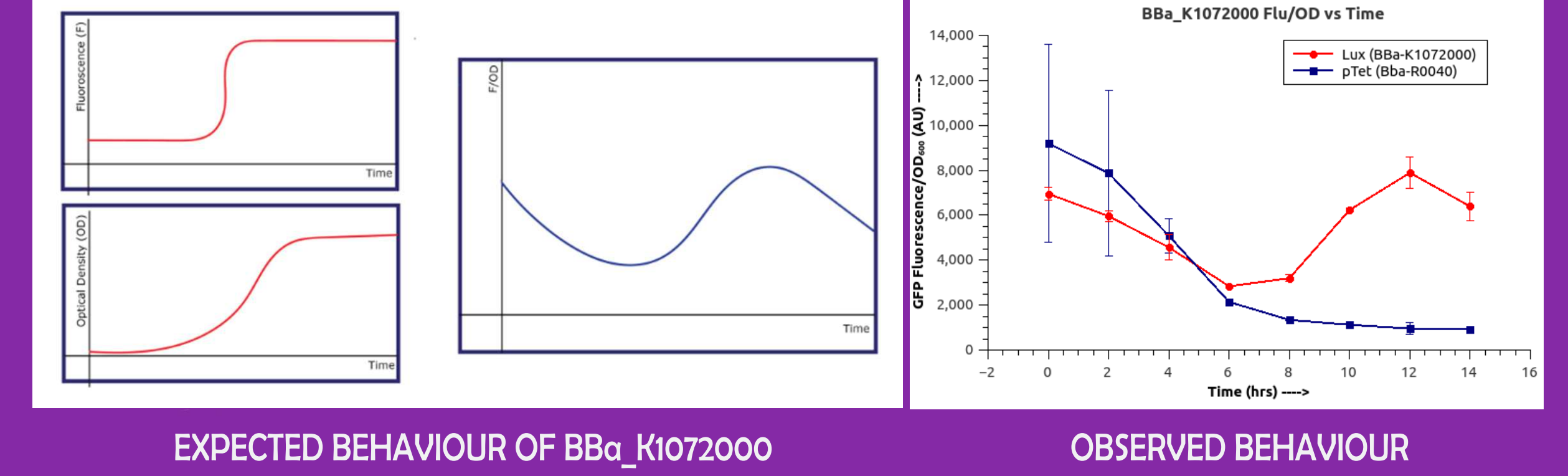


AUTO INDUCTION

Quorum sensing refers bacterial signalling mechanisms that regulate gene expression dependent on local cell density.

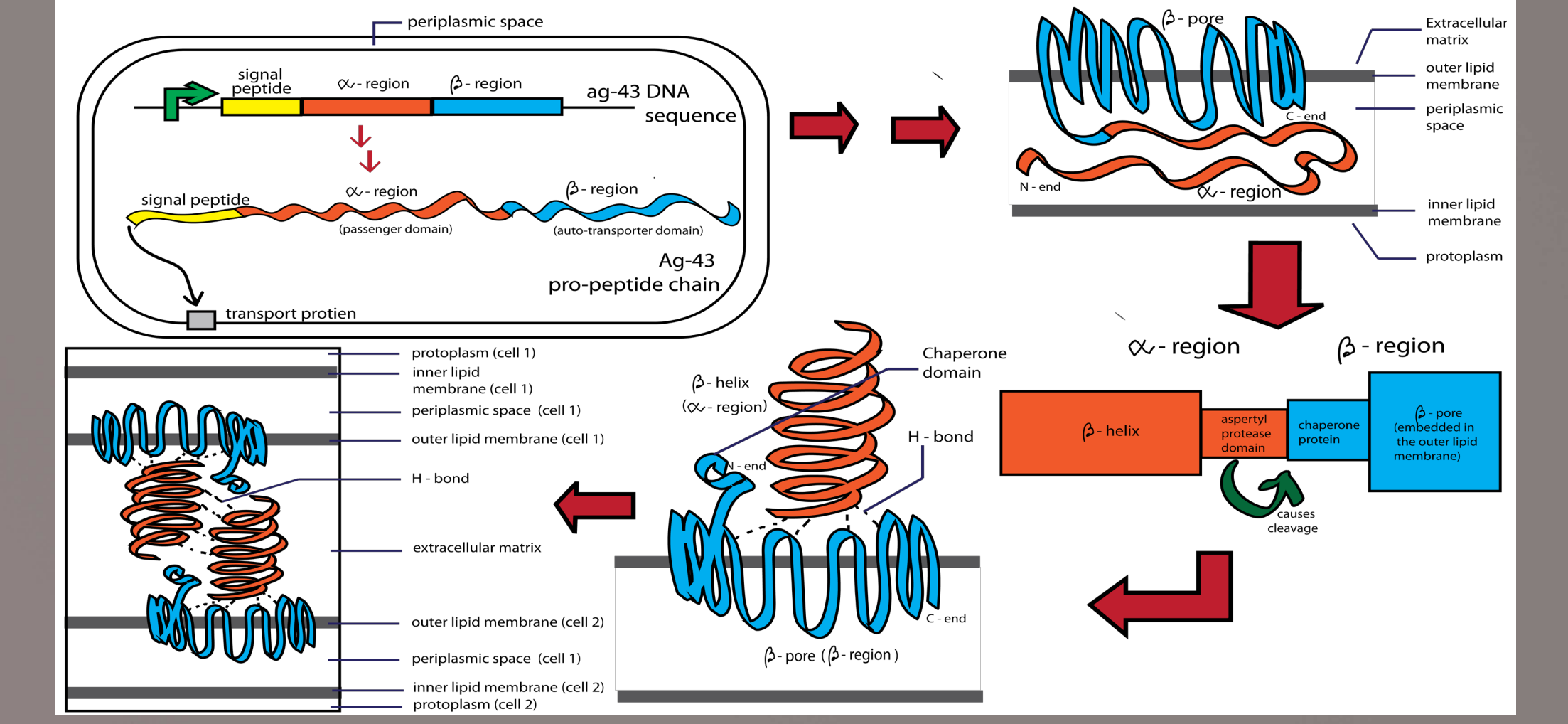


While components of quorum sensing have been explored in iGEM since 2004, no team has so far characterized the switching behaviour of the lux system.

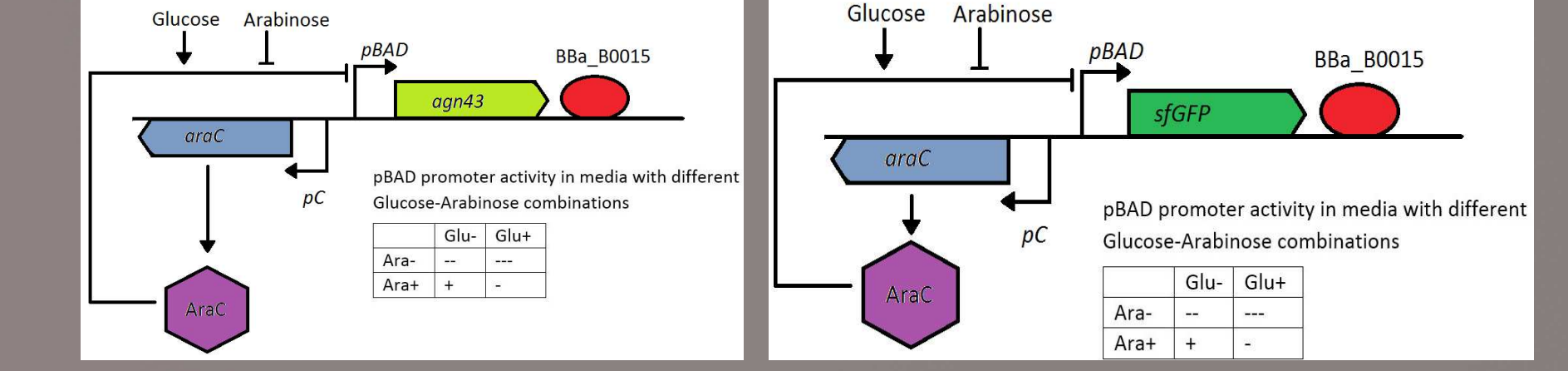


AUTO AGGREGATION

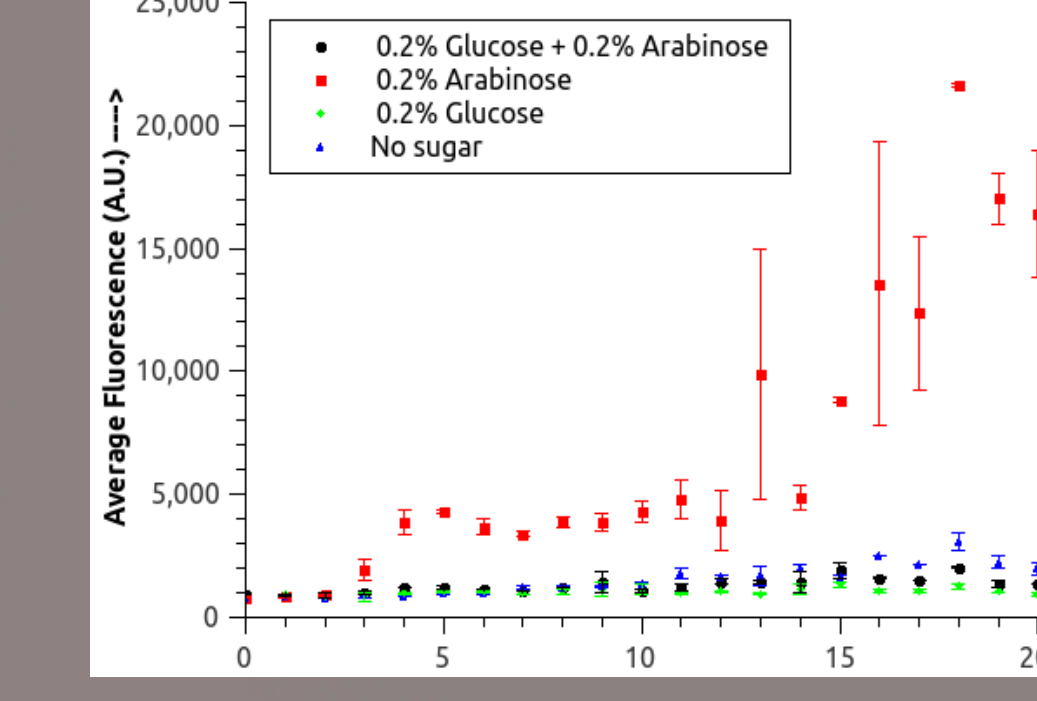
Ag43 (antigen 43) is an outer-membrane cell-cell adhesion protein native to *E.coli* K strains. It belongs to the type V secretion system family of outer membrane proteins and can form a homodimer.



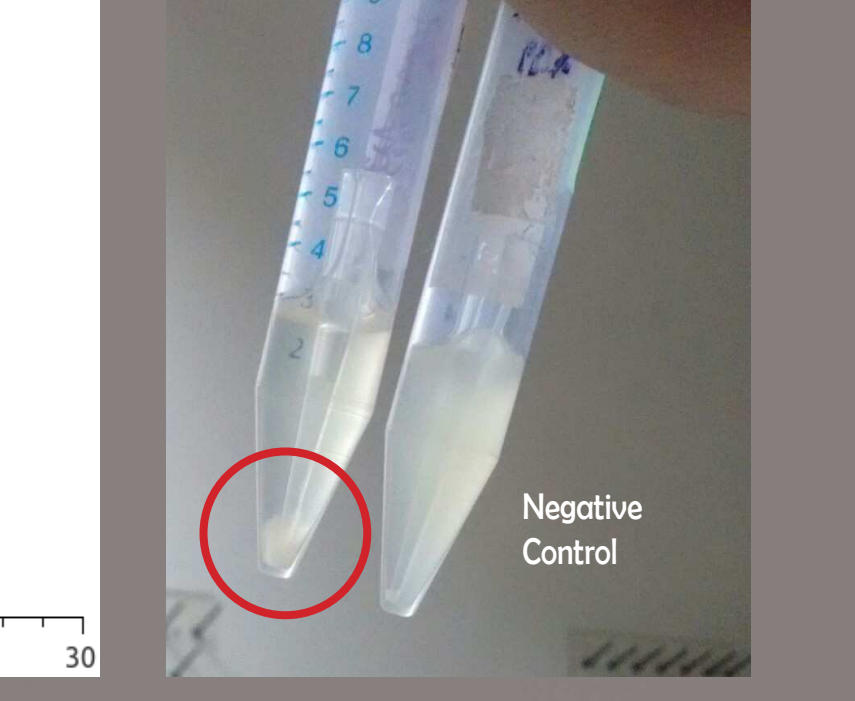
Gene regulation by pBAD/araC



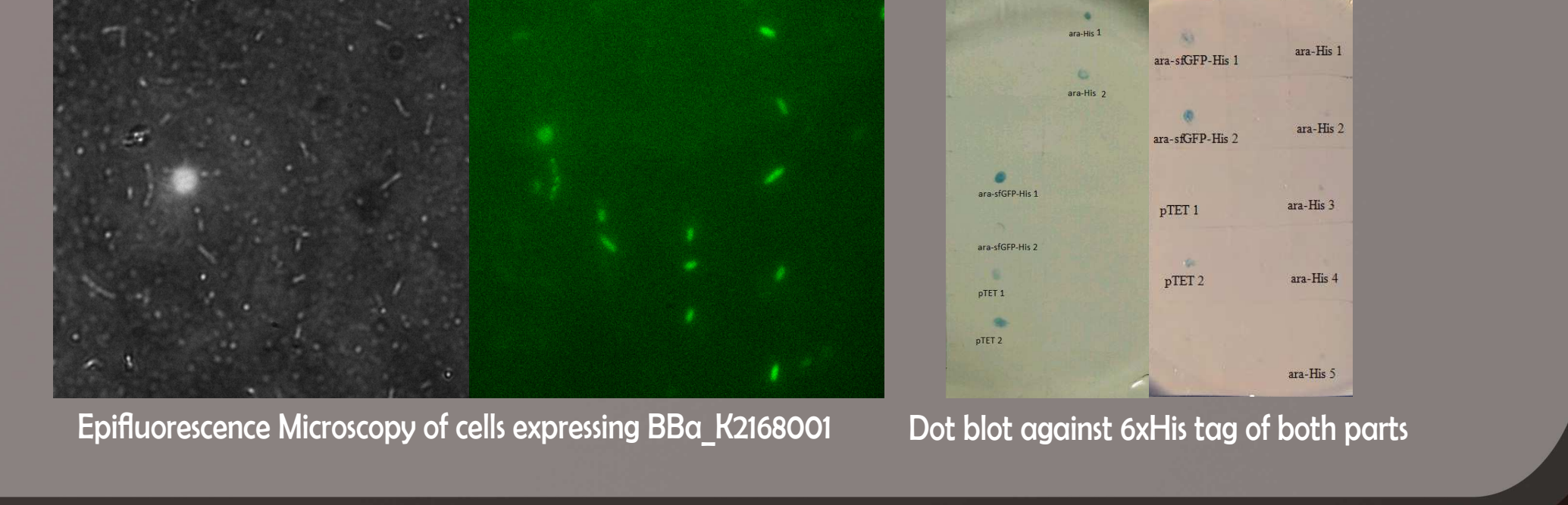
Growth Curve of BBa_J746908 in Different Media



Aggregation by cells expressing Ag43 (left)



Characterization of BBa_K2168000 and BBa_K2168001



HUMAN PRACTICES

PRESENTATIONS IN SCHOOLS



MEETING WITH THE GOVERNMENT OF KARNATAKA

1. Meeting with officials in Dept. of IT, BT & S&T.
2. Intent: convince them of the need for enhancing government biotech parks
3. Novelty of use of Ag43 and autoinduction to replace machinery - patent worthy
4. \$9,000 grant received, message delivered to government.

PRESENTATION IN IISc UG SCIENCE CLUB

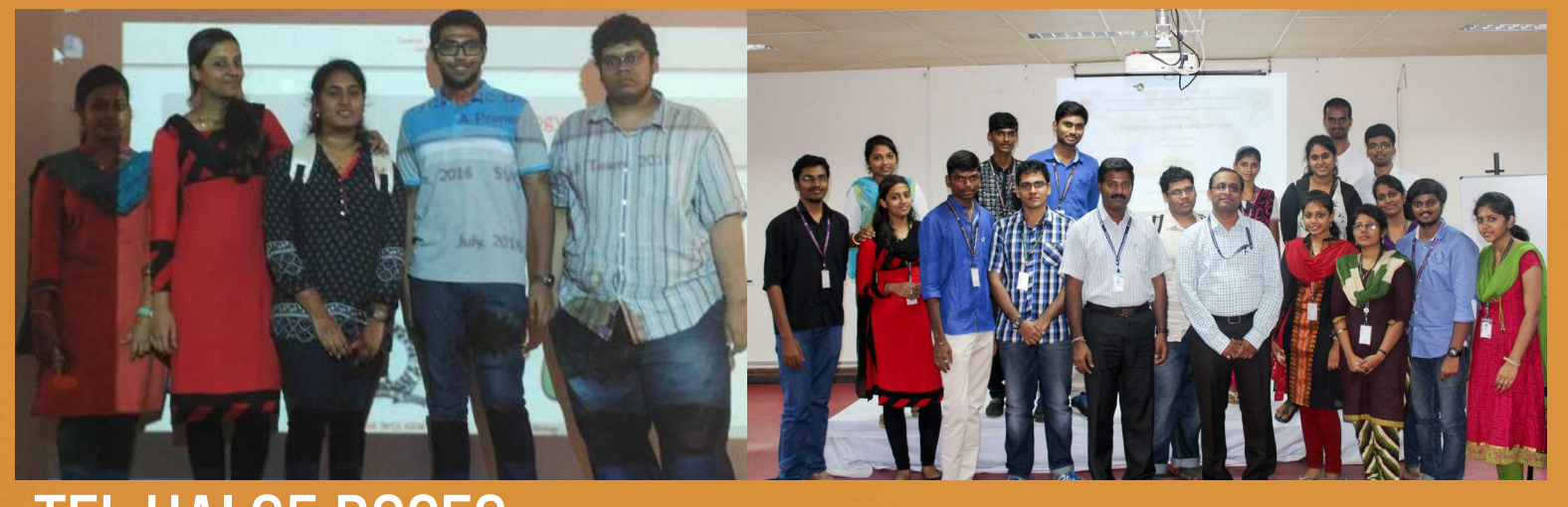


COLLABORATIONS

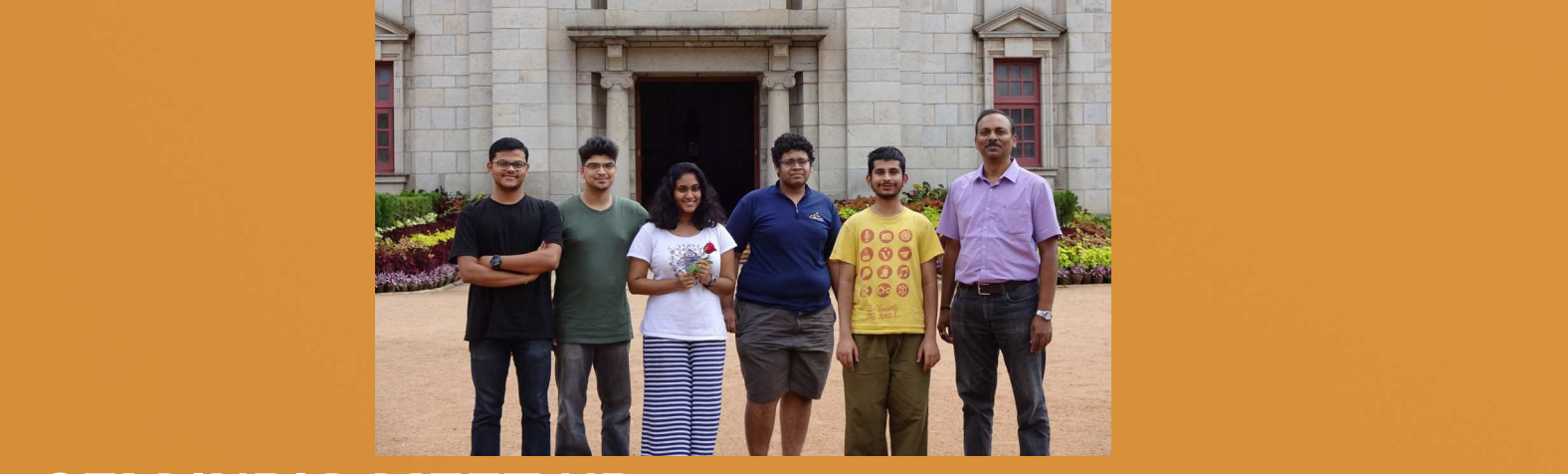
IIT MADRAS

We added T7 promoter (BBa_K525998) to regulate GFP in IITM measurement device (BBa_K1956046). Principle of measurement device: replaceable promoters and RBSs with fluorescent reporters regulated by the same. Above part was characterized, to verify the functioning of the device.

SVCE COLLABORATION



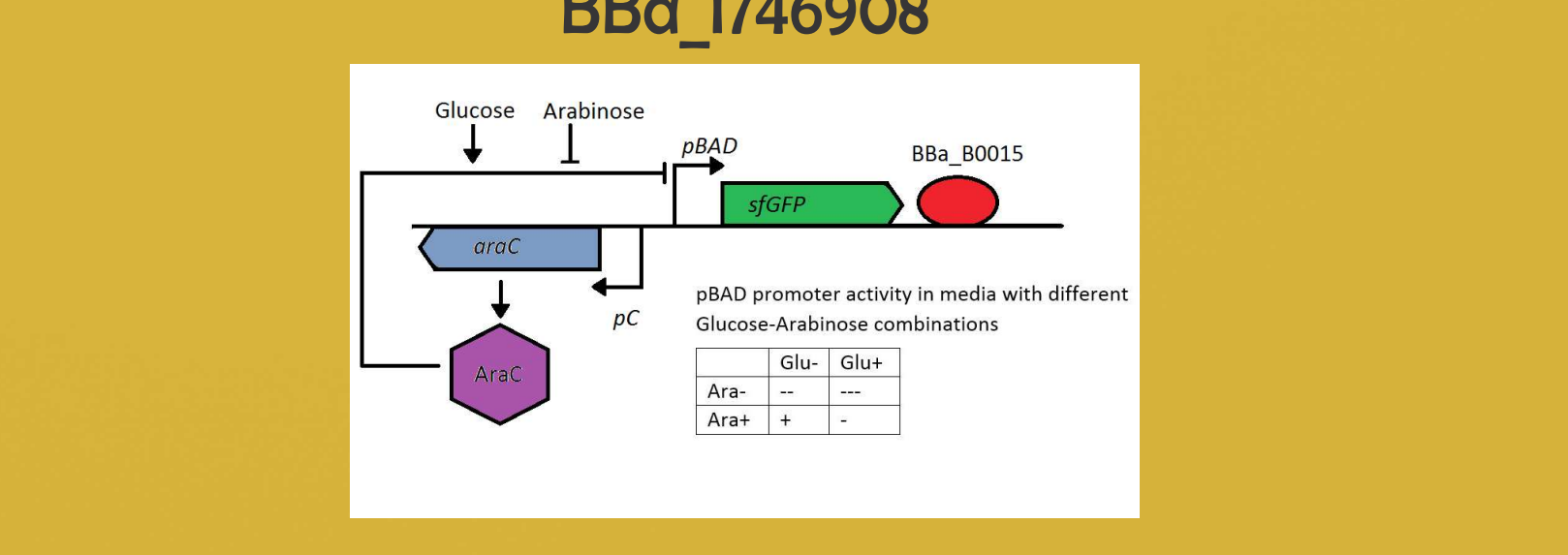
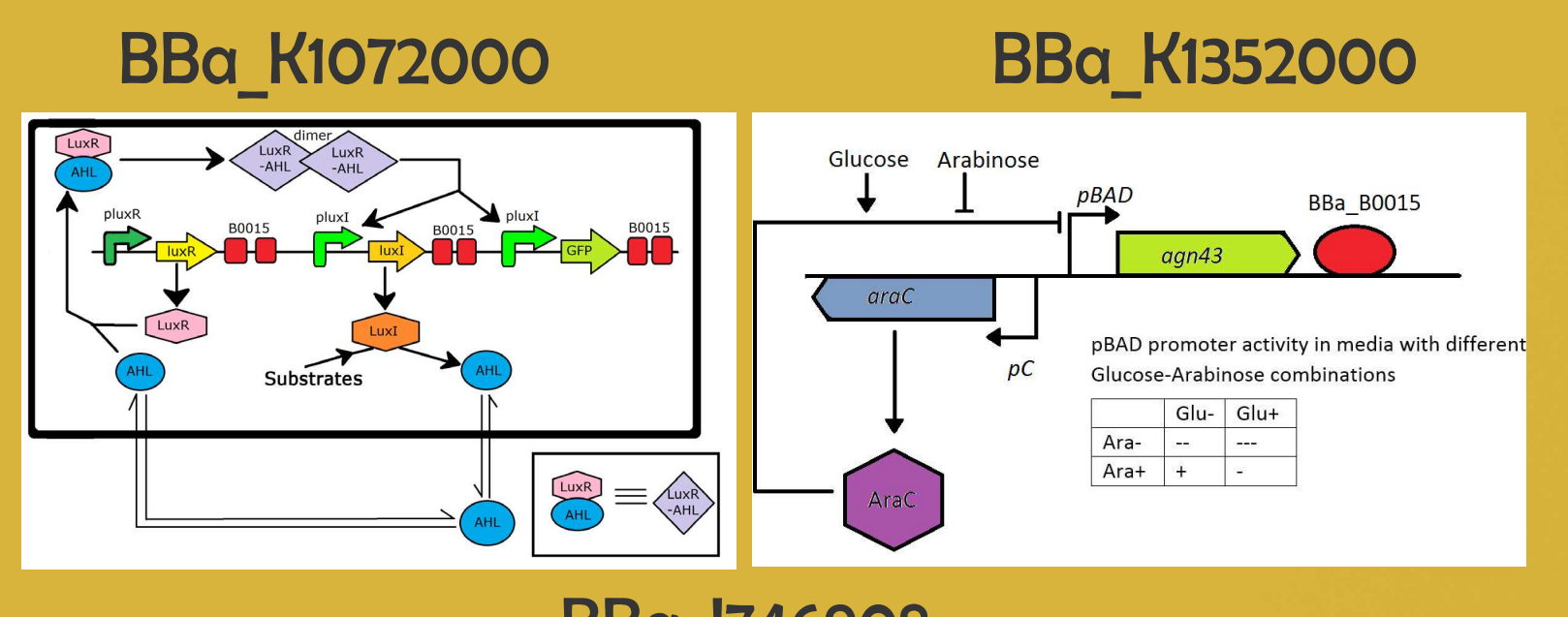
TEL HAI 65 ROSES



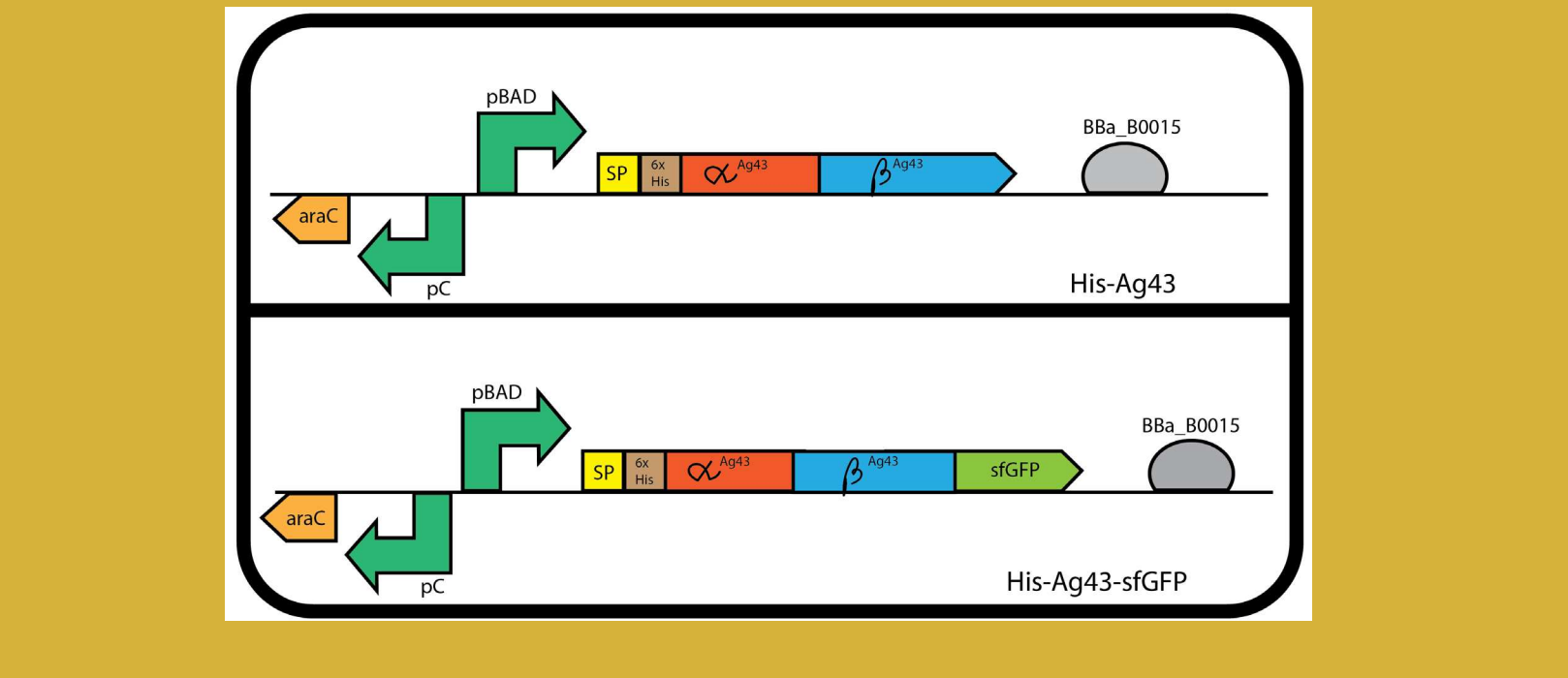
iGEM INDIA MEET UP



PARTS USED



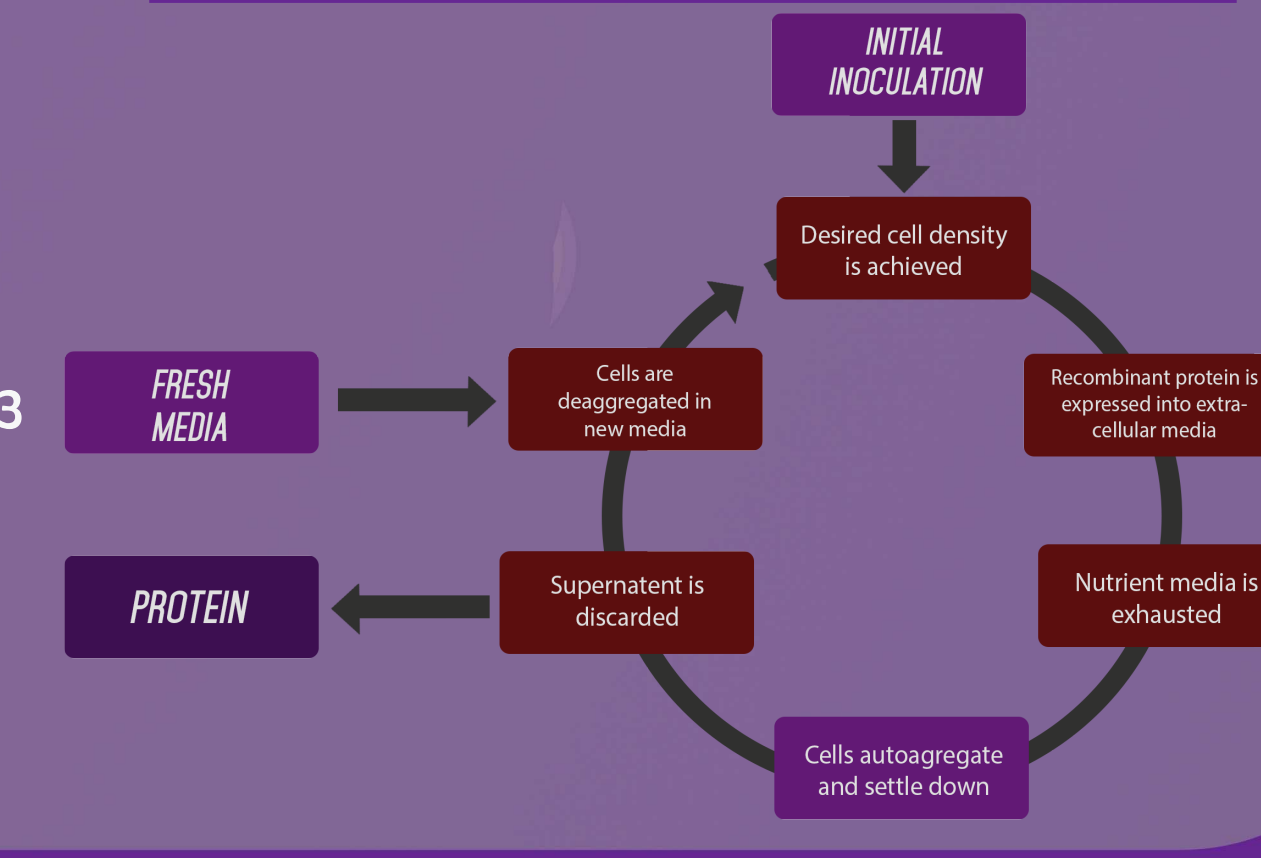
Above: BBa_K2168000 and Below: BBa_K2168001



FUTURE DIRECTIONS

During our summer, we realized several ways to improve on our project:

- 1) Test different Quorum Sensing systems to maximize yield
- 2) Designing a regulation mechanism for Ag43 independent of the media, such as a light based method.
- 3) Developing Ag43 mutants for stronger aggregation.



SPONSORS



REFERENCES

1. Heras B, Totsika M, Peters KM, et al. The antigen 43 structure reveals a molecular Velcro-like mechanism of autotransporter-mediated bacterial clumping. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(1):457-462. doi:10.1073/pnas.1311592111.
2. Kjargaard, K., Schembri, M. A., Hasman, H., & Klemm, P. (2000). Antigen 43 from *Escherichia coli* Induces Inter- and Intraspecies Cell Aggregation and Changes in Colony Morphology of *Pseudomonas fluorescens*. *Journal of Bacteriology*, 182(17), 4789-4796. doi:10.1128/Jb.182.17.4789-4796.2000
3. Felo, M., Christensen, B., & Higgins, J. (2013). Process cost and facility considerations in the selection of primary cell culture clarification technology. *Biotechnology Progress*, 29(5), 1239-1245. doi:10.1002/btpr.1776
4. Chu T, Ni C, Zhang L, et al. A quorum sensing-based in vivo expression system and its application in multivalent bacterial vaccine. *Microbial Cell Factories*. 2015;14:37. doi:10.1186/s12934-015-0213-9.

